



**HAL**  
open science

## Basophils drive the resolution and promote wound healing in adult and aged mice

Julie Bex, Victoria Peter, Chaimae Saji, Léa Chapart, Suoqin Jin, Gregory Gautier, Olivier Thibaudeau, Morgane K Thaminy, John Tchen, Quentin Simon, et al.

► **To cite this version:**

Julie Bex, Victoria Peter, Chaimae Saji, Léa Chapart, Suoqin Jin, et al.. Basophils drive the resolution and promote wound healing in adult and aged mice. 2024. hal-04758884

**HAL Id: hal-04758884**

**<https://hal.science/hal-04758884v1>**

Preprint submitted on 29 Oct 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Public Domain

1 **Basophils drive the resolution and promote wound healing in adult and aged mice**

2

3 Julie Bex<sup>1</sup>, Victoria Peter<sup>1</sup>, Chaimae Saji<sup>1</sup>, Léa Chapart<sup>1</sup>, Suoqin Jin<sup>2</sup>, Gregory Gautier<sup>1</sup>, Olivier  
4 Thibaudeau<sup>3</sup>, Morgane K. Thaminy<sup>1</sup>, John Tchen<sup>1</sup>, Quentin Simon<sup>1</sup>, Xing Dai<sup>4</sup>, Kensuke Miyake<sup>5</sup>, Hajime  
5 Karasuyama<sup>5</sup>, Jean X. Jiang<sup>6</sup>, Karmella Naidoo<sup>7</sup>, Marc Benhamou<sup>1</sup>, Ulrich Blank<sup>1</sup>, Renato Monteiro<sup>1</sup>,  
6 Graham Le Gros<sup>7</sup>, Nicolas Charles<sup>1</sup>, Christophe Pellefigues<sup>1,7,8\*</sup>

7 1- Centre de Recherche sur l'Inflammation, INSERM UMR1149, CNRS EMR252, Faculté de Médecine  
8 site Bichat, Université Paris Cité, Laboratoire d'Excellence Inflammex, 75018, Paris, France

9 2- School of Mathematics and Statistics, Wuhan University, 430072 Wuhan, China

10 3- Université de Paris, INSERM, UMRS1152, Plateforme de Morphologie, 75018 Paris, France

11 4- Department of Biological Chemistry, School of Medicine, University of California, Irvine, USA

12 5- Inflammation, Infection and Immunity Laboratory, TMDU Advanced Research Institute, Tokyo  
13 Medical and Dental University (TMDU), Tokyo, Japan

14 6- Department of Biochemistry and Structural Biology, University of Texas Health Science Center, San  
15 Antonio, USA

16 7- Malaghan Institute of Medical Research, Victoria University, Wellington, New Zealand

17 8- RESTORE Research center, Université de Toulouse, INSERM UMR1301, CNRS EMR5070, EFS, ENVT,  
18 Toulouse, France

19 \*-Corresponding author. Current address: RESTORE Research center, Toulouse, FRANCE. Email:  
20 [Christophe.pellefigues@inserm.fr](mailto:Christophe.pellefigues@inserm.fr). <https://orcid.org/0000-0001-7036-0763>

21

22 **SUMMARY**

23 The resolution of inflammation is essential for homeostasis and becomes defective with age. Here we  
24 show basophil immunoregulatory properties promote resolution during skin wound healing, in both  
25 adult and aged mice.

26 **ABSTRACT**

27 An active resolution is critical to control the duration of inflammation and limit its pathological  
28 consequences. Defects in resolution during wound healing allow the emergence of chronic wounds, a  
29 common complication in the elderly. Here, we show that basophils infiltrate the periphery of mouse  
30 skin wounds for at least three weeks, during both the inflammation and resolution phases of wound  
31 healing. Depletion of basophils induces an increased secretion of inflammatory molecules,  
32 accumulation and activation of pro-inflammatory leukocytes, and delays the wound healing response.  
33 Basophils particularly promote epidermal differentiation towards homeostasis in the wounds.  
34 Basophil-derived IL-4 and M-CSF drive partly their immunoregulatory and healing properties.  
35 Unexpectedly, aged mice basophils infiltrate more potently the wounds to promote inflammation  
36 resolution, showing a transcriptomic signature biased towards tissue remodeling. Thus, basophils are  
37 not pro-inflammatory but pro-resolution cells during an essential biological process such as skin wound  
38 healing. Unraveling basophil pro-resolution properties may reveal new strategies to fight chronic  
39 wounds in the elderly.

40

## 41 Introduction

42 The delicate balance between pro-inflammatory, anti-inflammatory, and pro-resolution mechanisms  
43 during an inflammatory response needs to be coordinated to avoid excessive amplitude, duration, and  
44 pathological consequences in response to danger or damage. Uncontrolled inflammation can promote  
45 cytokine storms, fibrosis, or chronic wounds, especially in an increasingly fragile proportion of the  
46 population suffering from chronic inflammation such as the elderly. Immune cells are important actors  
47 in initiating, amplifying, or resolving inflammation during both infectious and sterile physiological  
48 responses(Furman *et al.*, 2019).

49 Basophils are rare circulating “type 2” granulocytes involved in allergic or autoimmune diseases and  
50 immunity against various parasites, known for their pro-inflammatory effector functions, and their  
51 pro-Th2 or Th17 immunomodulatory functions. However, basophils manifest both pro-inflammatory  
52 and pro-resolving properties in models of chronic skin allergic inflammation(Egawa *et al.*, 2013) or  
53 acute atopic dermatitis(Pellefigues, Naidoo, *et al.*, 2021). They can control the extent or amplitude of  
54 inflammation through various mechanisms(Miyake, Ito and Karasuyama, 2022; Poto *et al.*, 2023),  
55 including extracellular ATP degradation(Tsai *et al.*, 2015) and secretion of anti-inflammatory mediators  
56 such as retinoic acid(Hachem *et al.*, 2023), IL-10(Kleiner *et al.*, 2021), or the type 2 cytokines IL-4 and  
57 IL-13 (Pellefigues, Mehta, *et al.*, 2021). Basophils are the main source of IL-4 in models of helminth  
58 infection(van Panhuys *et al.*, 2011), atopic dermatitis(Pellefigues, Naidoo, *et al.*, 2021; Leyva-Castillo  
59 *et al.*, 2022; Takahashi *et al.*, 2023) or skin infection(Leyva-Castillo *et al.*, 2021). *In vivo*, basophil-  
60 derived IL-4 is sufficient to dampen epidermal type 17 inflammation against infections(Leyva-Castillo  
61 *et al.*, 2021). It also fosters the emergence of immunosuppressive myeloid cells in the bone  
62 marrow(LaMarche *et al.*, 2024) and promotes an M2-like macrophage bias and the resolution of  
63 inflammation in various experimental models of chronic skin allergic or atopic inflammation(Egawa *et al.*,  
64 2013; Pellefigues, Naidoo, *et al.*, 2021; Miyake *et al.*, 2024), liver infection(Blériot *et al.*, 2015) and  
65 heart infarction(Sicklinger *et al.*, 2021). Type 2 cytokines particularly promote monocyte-to-resident  
66 macrophage transition(Finlay *et al.*, 2023), local macrophage proliferation(Jenkins *et al.*, 2011) and  
67 protect macrophages from immunosenescence to dampen age-associated inflammation and  
68 frailty(Zhou *et al.*, 2024). Basophils also secrete M-CSF, a growth factor critical for macrophage  
69 homeostasis. Basophil-derived M-CSF seems important for alveolar macrophage development(Cohen  
70 *et al.*, 2018) and promotes the resolution of skin atopic inflammation(Pellefigues, Naidoo, *et al.*, 2021).  
71 M-CSF works alongside IL-4 to regulate resident macrophage homeostasis *in vivo*(Jenkins *et al.*, 2013).  
72 In the skin, basophils promote keratinocyte differentiation and homeostasis in a model of epidermal  
73 inflammation(Strakosha *et al.*, 2024) and during atopic-like inflammation(Pellefigues, Naidoo, *et al.*,  
74 2021). As basophils express a very high diversity of ligands to communicate with both immune and  
75 non-hematopoietic cells(Cohen *et al.*, 2018; Cui *et al.*, 2024), they represent unique players to fine-  
76 tune the resolution of inflammatory events.

77 Here, we studied the role of basophils during both the inflammation and resolution phases in mouse  
78 models of wound healing. We found that basophils quickly infiltrated the periphery of skin wounds,  
79 and persisted for at least three weeks. During both the inflammatory and resolution phases,  
80 constitutive or conditional depletion of basophils led to an accumulation of pro-inflammatory  
81 mediators and immune cell activation, delayed differentiation of wound keratinocytes and wound  
82 closure. Basophil-derived IL-4 and M-CSF showed complex immunoregulatory properties in the  
83 wounds but IL-4 was critical for a monocyte to macrophage transition during the resolution phase. As  
84 resolution becomes defective with aging, we also analyzed the pro-resolution capabilities of basophils  
85 in aged mice. Unexpectedly, aged mice showed an increased basophil infiltration, which kept their pro-

86 resolving capabilities. This was confirmed by the reanalysis of a recently published scRNAseq dataset  
87 collected on mouse back skin wounds (Vu *et al.*, 2022). Overall, basophils drive the resolution of  
88 inflammation and accelerate skin wound healing, and these properties remain potent upon aging.

89

## 90 Results

### 91 1) Basophils infiltrate skin wounds early during inflammation and are activated during the 92 resolution phase coinciding with the emergence of M2-like macrophages

93 We used 2mm circular sterile punch biopsies to generate incisional wounds in mouse ears (“Ear punch”  
94 model) and monitored the kinetics of the local skin leukocyte infiltrate by flow cytometry. We observed  
95 an early significant peak of leukocyte accumulation at 24h, followed by their progressive  
96 disappearance during the three weeks of the study. Consensual pro-inflammatory cells such as  
97 neutrophils, Ly6C+ monocytes, and Ly6C+ macrophages exhibited similar kinetics and their proportions  
98 strongly decreased at one week post wound. More than 90% of macrophages expressed Ly6C on Day  
99 1. Another population of monocytes expressing CD16.2 (coded by *Fcgr4*), and eosinophils, did not show  
100 any enrichment during the course of the study. We also observed an early accumulation of basophils  
101 among skin leukocytes on Day 1 but contrary to the kinetics of pro-inflammatory cells, it remained  
102 significant for 3 weeks (Figure 1A, B). The peak of neutrophil accumulation can be used to define the  
103 onset of the resolution phase, which occurred between Day 1 and 7 in this model. These kinetics were  
104 confirmed by the quantification of pro-inflammatory cytokines (IL-6, TNF $\alpha$ ) and monocytes attracting  
105 chemokines (CCL2, -3, -4) in the skin (Figure 1C). We did not detect any significant increase in  
106 interferons (IFN $\alpha$  and  $\gamma$ ), usually associated with anti-viral or anti-bacterial Th1 responses, which  
107 supports that the wound healing response developed in the absence of any infection in this model.  
108 Conversely, IL-4 levels, associated with Th2 responses, increased during the resolution phase (Figure  
109 1C). Th2 cytokines induce M2-like macrophages known to drive the resolution of inflammation and  
110 tissue repair. In concordance, the expression of genes related to M2-like macrophages (*Arg1*, *Retlna*),  
111 tissue repair macrophages (*Mgl2*), or the resolution (*Alox15*) tended to increase during the resolution  
112 phase (Supplementary Figure 1).

113 We confirmed these results in a second model of sterile wound healing induced by a 2mm plier-style  
114 ear punch (used commonly for ear tagging), which induces a laceration but not a clean incision of the  
115 tissue (Rajnoch *et al.*, 2003). This model showed similar yet delayed leukocyte infiltration kinetics  
116 which include an increased accumulation of basophils during the resolution phase (Supplementary  
117 Figure 2A). The resolution was characterized by an increase in M2-like macrophages expressing PDL2  
118 and CD206 (Figure 1D). PDL2+ pro-resolution macrophages are known to differentiate from Ly6C+  
119 inflammatory monocytes in response to basophil-derived IL-4 in the skin (Egawa *et al.*, 2013; Miyake *et al.*,  
120 2024). Several immune cell types expressed IL-4 and/or IL-13 in the skin during the wound healing  
121 response, including mast cells, eosinophils, CD4+ T cells, and innate lymphoid cells, but their  
122 proportion or expression of type 2 cytokines did not evolve with inflammation or resolution kinetics  
123 (Supplementary Figure 2B). On the contrary, basophil IL-4 expression was strongly associated with the  
124 onset of the resolution phase (Figure 1D).

125

126 We then assessed by confocal microscopy where basophils locally infiltrated the skin during wound  
127 closure in the ear punch model (Figure 2A). We observed a strong presence of MCPT8+ basophils in  
128 the ear skin at D1 post-wound (Figure 2B). However, they were not accumulating as close to the  
129 wound edge as Ly6G+ neutrophils, or CD68+ monocytes/macrophages, but they rather settled at a  
130 certain distance instead, at a median of 670.1 $\mu$ m from the lesion (Figure 2B-D). We also confirmed  
131 the presence of numerous basophils in the ear skin by microscopy at D7 and D21 after injury (Figure  
132 2E-F).

## 133 **2) Basophils drive the resolution during the inflammation phase**

134 Next, we studied the roles of basophils during the inflammatory phase of the wound healing response  
135 in the ear punch model by conditionally depleting them by injection of diptheria toxin (DT) in MCPT8-  
136 DTR mice. One day after wounding, basophil-specific depletion did not alter the skin immune infiltrate  
137 significantly (Figure 3A), except for an increased recruitment of CD16.2+ monocytes (Figure 3B).  
138 However, in the absence of basophils, monocytes, and macrophages exhibited a more activated  
139 phenotype: they expressed more CD11b (integrin and complement receptor), CD64 (activating high-  
140 affinity IgG receptor), and/or were enlarged (Figure 3B), and the skin microenvironment was more  
141 inflammatory and contained more pro-inflammatory cytokines and monocyte/macrophages attracting  
142 chemokines including IL-6, CCL2 and CCL4 (Figure 3C). As shown in Figure 1, there was a substantial  
143 decrease in both the leucocytic and the neutrophilic infiltrate one week after wounding, indicating the  
144 early resolution phase in this particular model. Basophil depletion from D-2 to D7 led to an increased  
145 accumulation of pro-inflammatory leukocytes in the wounds, including neutrophils and Ly6C+  
146 macrophages without impacting the overall leucocytic infiltrate (Figure 3D). Moreover, neutrophils,  
147 Ly6C+ and CD16.2+ monocytes, and Ly6C+ macrophages showed increased activation markers and  
148 were all enlarged after basophil depletion (Figure 3E). Similarly, basophil depletion led to an  
149 accumulation of pro-inflammatory mediators at the onset of the resolution phase, including CCL2 and  
150 CXCL10 (Figure 3F). Overall, these results indicated that basophils dampen the activation of pro-  
151 inflammatory cells and wound inflammation during both the inflammation phase and the early  
152 resolution phase.

## 153 **3) Basophils promote keratinocyte differentiation and the wound healing response**

154 Uncontrolled inflammation has been associated with delayed wound healing response and the  
155 emergence of chronic wounds (Krzyszczuk *et al.*, 2018). The ear biopsy punch model allows to quantify  
156 wound closure kinetics by monitoring the ear area that remained open. Of note, we observed different  
157 kinetics depending on the status ("Conventional or Specific Pathogen Free") of the animal facility with  
158 an increased wound closure in conventional facilities (Figure 4A). As basophils promoted a resolution  
159 environment, we explored if basophils were affecting the kinetics of wound closure. Indeed, mice  
160 constitutively deficient in basophils showed delayed wound closure during the second week of healing  
161 (Figure 4B). Similarly, specifically depleting from D-2 to D7 after wounding significantly and transiently  
162 decreased wound closure in the first week (Figure 4C).

163 As basophils are known to control keratinocyte differentiation during skin inflammation (Hayes *et al.*,  
164 2020; Pellefigues, Naidoo, *et al.*, 2021; Strakosha *et al.*, 2024), and the inflammatory processes in the  
165 wounds as shown here (Figure 3), we explored their roles in controlling reepithelialization in the ear  
166 punch model. Reepithelialization occurs via the proliferation, migration, and differentiation of  
167 keratinocytes, which will quickly cover the wound area by forming a "migrating epidermal tongue"  
168 from the adjacent epithelium which show increased proliferation and thickening. At 24h the wounds  
169 were never covered by keratinocytes in this model (Figure 4D). The thickness of the adjacent epidermis  
170 reflects the initial rate of keratinocyte differentiation and proliferation and the length of the migrating

171 epithelial tongue can be measured as a surrogate for the rate of the reepithelialization(Nascimento-  
172 Filho *et al.*, 2020; Bornes *et al.*, 2021). The presence of basophils in the wounds at Day 1 was redundant  
173 for these phenomena (Figure 4D).

174 In this model, wounds were always covered by keratinocytes at D4, with a very heterogeneous  
175 epidermal thickness. The wound bed epidermis thickened heterogeneously until reaching a plateau at  
176 D7, before progressively returning to homeostasis levels (Figure 4E). Basophil depletion during the first  
177 week of healing led to increased inflammation and thickening of the wound bed epidermis at D7  
178 (Figure 4F, G).

179 During homeostasis, Keratin 14+ keratinocytes (K14+) constantly proliferate in a “*stratum basale*”  
180 layer, before differentiating into K10+ keratinocytes (*Stratum spinosum*) and then expressing filaggrin  
181 (FLG+) in granules (*Stratum granulosum*), before dying by desiccation (*Stratum corneum*). This  
182 controlled physiological differentiation of epidermal keratinocytes is altered during wound healing,  
183 with an increased proliferation and differentiation of keratinocytes in both the wound bed and the  
184 adjacent epidermis (Figure 4G). Non-healing chronic wounds show a defect in keratinocytes terminal  
185 differentiation and of their expression of both K10 and Flg, and they stay in a hyperproliferative  
186 state(Stojadinovic *et al.*, 2008; Wikramanayake, Stojadinovic and Tomic-Canic, 2014). Here, one week  
187 post-wound, the epidermis of basophil-depleted mice was less differentiated: the wound bed  
188 contained fewer FLG+ cells, while the adjacent epithelium contained less K10+ but more K14+  
189 keratinocytes (Figure 4H).

190 Thus, basophils accelerate skin wound closure and promote the differentiation of keratinocytes during  
191 the resolution phase of wound healing, but are redundant for the initial wound reepithelialization.

192

#### 193 **4) Basophils actively drive the resolution during the resolution phase**

194 To decipher the role of basophils in controlling the immune response during the resolution phase of  
195 wound healing, we analyzed the skin infiltrate of mice constitutively deficient in basophils (“Baso-KO”),  
196 at 3 weeks post-wound (D21) in the ear punch model. While these mice did not show an aberrant  
197 quantity of leukocyte infiltration, basophil depletion led to a significant reorganization of the infiltrate,  
198 containing more pro-inflammatory cells, including neutrophils and Ly6C+ monocytes at the expense of  
199 non-inflammatory Ly6C- macrophages (Figure 5A). As this accumulation of pro-inflammatory cells  
200 during the resolution phase could be caused by the absence of basophils during the inflammatory  
201 phase (Figure 3), we used MCPT8-DTR mice to deplete basophils during only the 3<sup>rd</sup> week of the wound  
202 healing response (Figure 5B). This conditional depletion, like the constitutive depletion, led to an  
203 accumulation of pro-inflammatory cells in the skin, including neutrophils and Ly6C+ macrophages, but  
204 not Ly6C+ monocytes (Figure 5C) or CD16.2+ monocytes (Supplementary Figure 3A). In addition, it led  
205 to the activation of wound neutrophils, Ly6C+ monocytes, and macrophages, which showed an  
206 increased expression of CD11b, CD16.2, CD64, and/or were enlarged. Ly6C- non-inflammatory  
207 macrophages did not show this activated phenotype after basophil depletion (Figure 5D). Importantly,  
208 this also led to an accumulation of CCL2, a pro-inflammatory chemokine involved in the recruitment  
209 and activation of Ly6C+ monocytes (Figure 5E). Unexpectedly, basophil conditional depletion did not  
210 lead to any decrease in the expression of M2-like markers at the surface of Ly6C- non-inflammatory  
211 macrophages (Figure 5F).

212 As basophils are recruited early during the inflammatory phase but remained in high proportions in  
213 the wounds during the resolution phase, we explored if basophils were actively recruited to the  
214 wounds during the resolution phase. We depleted basophils transiently during the resolution phase

## Basophils drive wounds resolution

215 from D11 to D17 and then analyzed the skin immune infiltrate at D21 (Supplementary Figure 3B). Four  
216 days were sufficient for basophils to re-infiltrate the wounds to almost normal levels. No significant  
217 effect of their transient depletion on the accumulation of pro-inflammatory cells into skin wounds was  
218 observed (Supplementary Figure 3C). This suggests that basophils are actively recruited during both  
219 the inflammatory and the resolution phase into skin wounds to regulate transiently the immune  
220 environment.

221 Overall, these results show that basophils actively dampen inflammation during the late resolution  
222 phase of the skin wound healing response.

223

## 224 **5) Basophil-derived IL-4 and CSF1 promote inflammation resolution and wound healing**

225 As we previously showed that basophil-derived IL-4 and CSF1 were important for the resolution of skin  
226 allergic inflammation(Pellefigues, Naidoo, *et al.*, 2021), we then explored if they were important for  
227 their pro-resolution properties during skin wound healing. We specifically deleted IL-4(Tchen *et al.*,  
228 2022) or CSF1(Pellefigues, Naidoo, *et al.*, 2021) in basophils by Cre-Lox-specific recombination, and  
229 analyzed wound closure kinetics for 3 weeks. Here, basophil-specific depletion of CSF1 transiently  
230 delayed wound closure in the first week, while basophil-specific depletion of IL-4 delayed wound  
231 closure only during the third week (Figure 6A). This is in adequation with the fact that wound basophils  
232 only begin to express Il4 during the resolution phase (Figure 1D), while both blood and atopic skin  
233 basophils show a high constitutive expression of CSF1 (Uhlén *et al.*, 2015; Pellefigues, Naidoo, *et al.*,  
234 2021).

235 As basophil-derived CSF1 deletion delayed wound closure at D7, we analyzed its immunoregulatory  
236 role at this time point. Deleting CSF1 in basophils had no significant impact on the overall infiltration  
237 of leukocytes (Supplementary Figure 4A), but was associated with a minor increase of CD16.2  
238 expression by CD16.2+ monocytes (Supplementary Figure 4B).

239 We then analyzed the outcome of deleting CSF1 or IL-4 in basophils at three weeks post-wound, which  
240 had no significant impact on total leukocyte accumulation (Figure 6B). Deleting basophil CSF1 led to an  
241 accumulation of both Ly6C+ and Ly6C- macrophages in the skin at 3 weeks post-wound (Figure 6C).  
242 Furthermore, while it did not lead to any detectable change in Ly6C+ monocyte or macrophage  
243 activation, it increased the expression of the Fc receptor CD16.2 on CD16.2+ monocytes infiltrating the  
244 wounds (Figure 6D), as for Day 7 (Supplementary Figure 4B).

245 Deleting IL-4 in basophils promoted an accumulation of pro-inflammatory Ly6C+ monocytes in the  
246 wounds at D21, at the expense of both Ly6C+ and Ly6C- macrophage populations (Figure 6E). Both  
247 macrophage subsets showed a smaller size and lower CD11b expression in the absence of basophils  
248 IL-4, similar to Ly6C+ monocytes. Furthermore, basophil-derived IL-4 deletion increased CD64  
249 expression on both macrophage subsets but decreased it on Ly6C+ monocytes (Figure 6F). This was  
250 associated with a decrease of MHCII and CD206 expression, and an increase in CD301b expression, on  
251 Ly6C- macrophages, markers associated with M2-like polarization and wound healing (Figure 6G). This  
252 supports the known role of IL-4 on primary myeloid cells to downregulate CD64 and to upregulate  
253 CD11b and CD206 expression(Wong *et al.*, 1992; de Waal Malefyt *et al.*, 1993), and to promote  
254 macrophage differentiation from inflammatory monocytes(Finlay *et al.*, 2023; Miyake *et al.*, 2024).

255 Taken together, basophils show complex immunoregulatory properties and promote wound closure  
256 in part via their secretion of CSF1 and/or IL-4, albeit with different kinetics. Basophil secretion of IL-4  
257 was particularly associated with a monocyte-to-macrophage transition and an M2-like polarization in  
258 the wounds.

259

## 260 **6) Basophils are more activated and increased in skin wounds of aged mice**

261 Aging is associated with a low-grade chronic systemic inflammation termed  
262 “Inflammaging”(Franceschi *et al.*, 2018), and a dysfunction of the immune system involving in part  
263 defects in the resolution of inflammation. This defect in resolution is thought to be critical for the  
264 frequent development of chronic wounds observed in the elderly(De Maeyer *et al.*, 2020). As basophils  
265 were scarcely studied in aged mice(van Beek *et al.*, 2018), we explored the phenotype of blood  
266 leukocytes in 75 to 85 weeks old C57BL/6J mice (“Aged”). As expected, these mice tended to have



267 fewer circulating leukocytes (Figure 7A) and showed decreased proportions of lymphocytes at the  
268 benefit of circulating monocytes and granulocytes populations, including neutrophils, Ly6C+  
269 monocytes, CD16.2+ monocytes, and basophils (Figure 7B). Circulating basophils from aged mice  
270 displayed an increased activation status and notably showed increased IgE binding, without a  
271 detectable significant increase in the expression of the alpha chain of the high affinity receptor for IgE.  
272 Basophils in aged mice also showed an increased expression of CD11b and CXCR4 (chemokine receptor  
273 of CXCL12)(Pellefigues *et al.*, 2018) and tended to express more CD200R1. They also displayed a  
274 decreased expression of CD200R3, which decreases upon basophil activation (Iwamoto *et al.*, 2015;  
275 Pellefigues, Mehta, *et al.*, 2021). This increased activation of basophils due to aging was also observed  
276 in the skin 24h after wounding (Figure 7D).

277 After wounding basophils were more potently infiltrating the skin in aged mice, both during the  
278 inflammatory and the resolution phases (Figure 7E). In parallel, we detected a decreased infiltration of  
279 neutrophils while Ly6C+ monocytes were increased at 24h in aged mice. However, the proportion of  
280 neutrophils remained high at 7 days post-wound in aged mice (Figure 7E), supporting the known  
281 altered kinetics of neutrophil accumulation in the skin wounds of aged individuals(De Maeyer *et al.*,  
282 2020). As both basophil and neutrophil proportions were increased in the bloodstream of aged mice,  
283 we calculated the basophil-to-neutrophil ratio in skin wounds to appreciate the relative enrichment of  
284 basophils in the skin. This ratio showed a selective enrichment of basophils from the blood to the skin  
285 from day 1 to day 21 post-wound, and from adult to aged mice at days 1 and 21 (Figure 7F). Thus,  
286 aging is associated with an increased recruitment and activation of basophils during both the  
287 inflammation and resolution phases of wound healing.

288 Next, we sought to confirm these findings on another model of wound healing by reanalyzing our  
289 recently published scRNAseq dataset of the full-thickness dorsal wounds of adult (8 weeks old) and  
290 aged mice (88 weeks old) at days 0, 4, and 7(Vu *et al.*, 2022). A t-SNE unsupervised clustering allowed  
291 to discriminate a cluster showing high *Kit* expression (“Mast cells”), from a second cluster expressing  
292 *Mcpt8*, a specific marker for basophils (Figure 8A). Both clusters were the main sources of *Il4* in the  
293 skin, while *Il13* and *Csf1* showed a broader pattern of expression. The basophil cluster also contained  
294 most of the cells positive for *Hgf*, a growth factor important for inflammation resolution and wound  
295 healing(Rutella, 2006; Nishikoba *et al.*, 2020) and for *Alox15*, a lipoxygenase-producing specialized pro-  
296 resolution mediators (SPMs) and known marker of pro-resolution cells(Serhan, 2014). Basophils also  
297 expressed high levels of *Alox5ap*, an accessory protein fostering lipoxygenase activity, including SPMs  
298 production (Serhan, 2014) (Figure 8B). Analyzing the proportion of basophils in the wounds over time  
299 underlined that basophils infiltrate the wounds faster in aged mice (Figure 8C). As basophils are more  
300 activated early on in aged mice (Figure 7), we analyzed the kinetics of expressed genes related to  
301 resolution over time. At 4 days post-wound, basophils from aged mice tended to express more *Il4*, and  
302 expressed more *Csf1* than their adult counterpart, but showed a similar expression of *Hgf*. Importantly,  
303 the expression of *Ptgs2* (coding for COX2, an enzyme involved in lipid mediators and SPM production),  
304 and *Alox15* (whose expression is induced by type 2 cytokines(Serhan, 2014)), were mostly detected in  
305 wound basophils of aged mice (Figure 8D).

306 The early activation of basophils from aged mice could be due to an intrinsic property related to age.  
307 To test this hypothesis, we analyzed the main differential gene expression between wound basophils  
308 in adult and aged mice. Adult basophils expressed more genes related to endocytosis (*Vps37b*, *Cltc*,  
309 *Flnb*), cellular stress or inflammation (*Dnaja1*, *Hspa1b*, *Hnrnpul2*), regulation of transcription or  
310 traduction (*Sec61a1*, *Nr4a3*, *Jarid2*), and the receptor for common  $\beta$  chain cytokines (*Csf2rb*, *Csf2rb2*),  
311 which include IL-3, a growth factor and cytokine critical for basophils homeostasis. Interestingly, they  
312 also show an increased expression of *Ly6C2*, which we did not observe by flow cytometry at the protein

313 level in the ear punch model (Supplementary Figure 1A), but that can be induced in response to IL-3 *in*  
314 *vitro*. It was also observed in the skin in a model of atopic dermatitis(Pellefigues *et al.*, 2019). By  
315 contrast, basophils from aged mice were characterized by an increased expression of histone variants  
316 (*H3f3b*, *Hist1h2bc*, *Hist1h4i*), known to increase during cellular senescence(Dubey, Dubey and  
317 Kleinman, 2024) as well as genes coding proteins with anti-inflammatory properties such as thymosin  
318  $\beta$ 4 (*Tmsb4x*), peroxiredoxin 5 (*Prdx5*) (Philp and Kleinman, 2010; Knoops *et al.*, 2011) or I $\kappa$ B $\alpha$  (*Nfkbia*)  
319 (Pellefigues, Mehta, *et al.*, 2021). They also expressed more coactosin-like protein (*Ct11*), a chaperone  
320 stabilizing lipoxygenase activity (Basavarajappa *et al.*, 2014), CD16 (*Fcgr3*), known to be expressed by  
321 a minor subset of basophils in human blood(Vivanco Gonzalez *et al.*, 2020), and *Il18rap*, coding for a  
322 chain of the IL-18 receptor. IL-18 is an epidermal-derived alarmin known to potently activate basophils  
323 (Ferrucci *et al.*, 2005; Pellefigues, Mehta, *et al.*, 2021), which is increased in the plasma of both old  
324 humans and mice(Brigger *et al.*, 2020) (Figure 8E). We then focused on the differences between adult  
325 and aged wound basophils at 4 days post-wound, which showed increased activation in aged basophils.  
326 Gene ontology (GO) of biological processes revealed a bias towards T cell activation and regulation of  
327 leukocyte adhesion in adult mice, whereas basophils from aged mice were enriched for genes  
328 associated with remodeling and homeostasis (Figure 8F). Inference of putative cell-cell  
329 communications based on ligand and receptor gene expression revealed that basophils' primary  
330 targets were more likely to be neutrophils, monocytes, and macrophage subsets than other cell types  
331 in the wounds (Figure 8G). Furthermore, analyzing the intercellular signaling networks of IL-4 and CSF1  
332 revealed basophils and mast cells to be the predominant sources of IL-4 in aged mouse skin wounds,  
333 able to interact with multiple different cellular targets, whereas CSF1 expression was broader, but able  
334 to target mainly cells of the monocytic lineage, including monocytes, macrophages subsets, osteoclast-  
335 like cells, and dendritic cells (Figure 8H).

336 These results confirm that basophils infiltrate mouse skin wounds and display a unique  
337 immunoregulatory potential during the resolution of inflammation. Overall, while basophils from aged  
338 mice display some markers of senescence, they show a faster activation and ability to infiltrate the  
339 wounds. Their transcriptomic signature is biased toward tissue remodeling and homeostasis when  
340 compared to adult basophils in the wounds. This suggests that basophils' pro-resolution and pro-  
341 healing properties are not diminished but enhanced by aging.

342

## 343 **7) Basophils drive the resolution in wounds of aged mice**

344 We then analyzed the function of basophils during the inflammation, early resolution, and late  
345 resolution phase of wound healing in aged mice (75 to 85 weeks old) using the ear punch model.  
346 Depleting specifically basophils in aged mice did not change the overall leukocyte infiltrate at day 1  
347 (Figure 9A). However, it led to a dramatic change in the quantity of the infiltrate, with a strong rise in  
348 neutrophilic infiltration at the detriment of Ly6C<sup>+</sup> monocytes (Figure 9B). At this early time point,  
349 basophils from aged mice were dampening the activation of neutrophils, monocytes, and macrophages  
350 into the wound, as revealed by their increased activation phenotype (CD11b, Size, CD64, CD16.2) after  
351 basophil depletion (Figure 9C). Similarly, while basophil depletion during the first week did not change  
352 the quantity of the leukocyte infiltrate (Figure 9D), it promoted the accumulation of monocytes (Ly6C<sup>+</sup>  
353 and CD16.2+), Ly6C<sup>+</sup> pro-inflammatory macrophages (Figure 9E) and their activation alongside  
354 neutrophils (Figure 9F). Importantly, basophil depletion starting at D-2 led to a significant delay in ear  
355 wound closure at D7 (Figure 9G), indicating that basophils were accelerating wound healing in aged  
356 mice as well.

357 Next, we analyzed the role of basophils during the third week of the wound healing response in aged  
358 mice after their conditional specific depletion (Figure 9H). Basophil depletion did not lead to a

359 significant decrease in skin leukocyte content (Figure 9I) but promoted the accumulation of both Ly6C-  
360 and Ly6C+ macrophages in the wounds (Figure 9J). The depletion further led to an activation of the  
361 neutrophil and monocyte/macrophage compartments (Figure 9K) but did not induce an observable  
362 delay in wound closure. Of note, this last analysis was limited to aged males (Figure 9L). Importantly,  
363 we observed that wound closure was faster in aged versus adult female mice, as previously  
364 shown (Nishiguchi *et al.*, 2018), but also in aged females when compared to aged males  
365 (Supplementary Figure 5A).

366 Importantly, transiently depleting basophils from D10 to D17 in aged mice did not significantly  
367 decrease wound basophil numbers at D21, as in adult mice (Supplementary Figure 3D) suggesting that  
368 basophils quickly re-infiltrated the wounds during the resolution phase. This transient depletion led to  
369 a lasting accumulation of pro-inflammatory Ly6C+ macrophages and tended to increase the  
370 accumulation of neutrophils and Ly6C+ monocytes, in the wounds (Supplementary Figures 5B-D).

371 Basophils promoted an early neutrophil-to-monocyte transition in aged mice wounds before reducing  
372 monocyte and macrophage accumulation and activation for at least 3 weeks. This was associated with  
373 an acceleration of wound closure during the first week. Overall, basophils show pro-resolution and  
374 pro-healing properties in old mice wounds.

375

## 376 Discussion

377 Basophils are pro-inflammatory cells involved in allergic diseases and protection against helminths,  
378 type 2 immune responses involving IgE reactivity (Miyake, Ito and Karasuyama, 2022; Poto *et al.*, 2023).  
379 However, type 2 immune responses have also evolved as a response to tissue damage which regulates  
380 tissue remodeling and healing (Gieseck, Wilson and Wynn, 2018), and are well known to antagonize  
381 and counterbalance type 1 immune responses against pathogens, and their toxicity for the  
382 tissues (Gause, Wynn and Allen, 2013). Basophils are important players in this network. Besides  
383 antibody-dependent responses, they are also potently activated by innate immune mechanisms. They  
384 are particularly activated by epidermal-derived alarmins, which trigger the production of type 2  
385 cytokines (Pellefigues, Mehta, *et al.*, 2021), suggesting that they can participate in the response to  
386 tissue damage. Here, we demonstrate that basophils are strongly recruited to skin lesions in the  
387 absence of any noticeable infection. They display a particular wound infiltration kinetics: while pro-  
388 inflammatory leukocytes such as neutrophils infiltrate transiently the wounds during the inflammation  
389 phase, basophils accumulate in the wounds and persist through the resolution phase, as already shown  
390 in a model of atopic skin inflammation (Pellefigues, Naidoo, *et al.*, 2021). The fact that a basophil-to-  
391 neutrophil ratio is higher in the wounds than in the blood demonstrates the relative specificity of  
392 basophil infiltration in the wounds during the first three weeks of the healing process. We also show  
393 that basophils infiltrate the skin farther away from the wound edge than pro-inflammatory cells. This  
394 particular localization suggests their pro-resolution function may help to contain the spread of  
395 inflammation close to the wound site. In line with a pro-resolution role, basophil depletion led to an  
396 increased inflammation characterized by the accumulation of the chemokine CCL2 and pro-  
397 inflammatory leukocytes expressing its receptor CCR2 (including neutrophils and classical monocytes),  
398 during both the inflammation and resolution phases. In agreement, basophils were previously shown  
399 to inhibit neutrophil recruitment during skin infection (Leyva-Castillo *et al.*, 2021), epidermal  
400 abrasion (Strakosha *et al.*, 2024), or atopic-like skin inflammation and resolution (Pellefigues, Naidoo,  
401 *et al.*, 2021). Thus, it is tempting to speculate that basophil accumulation participates in shaping  
402 chemokine gradients around the wounds, thereby fine-tuning the recruitment of pro-inflammatory  
403 leukocytes at the inflammatory site and limiting unnecessary damage to the surrounding tissue.

404 Our data suggests that basophils accelerate ear wound closure between one and two weeks post-  
405 wound. Several biological processes participate in the regulation of the wound healing response,  
406 including hemostasis, re-epithelialization, angiogenesis, and tissue remodeling. As basophils are  
407 potentially activated by epithelial-derived signals(Pellefigues, Mehta, *et al.*, 2021), and regulate  
408 keratinocyte homeostasis in various models(Hayes *et al.*, 2020; Leyva-Castillo *et al.*, 2021; Pellefigues,  
409 Naidoo, *et al.*, 2021; Strakosha *et al.*, 2024), we focused on their role in the reepithelialization of skin  
410 wounds. Basophils did not control the initial phases of reepithelialization, known to be driven by IL-  
411 17A inflammation(Konieczny *et al.*, 2022). Instead, we demonstrate that basophils are accelerating  
412 keratinocyte terminal differentiation in the wound bed during the resolution phase, notably at the  
413 time at which they promote wound closure. This is in concordance with recent observations that  
414 basophils promote keratinocyte differentiation, including their expression of the genes *Krt10* and *Flg*,  
415 by counteracting IL-17A-driven inflammation during the recovery phase of experimental epidermal  
416 inflammation(Strakosha *et al.*, 2024). We previously described the role of basophils in promoting  
417 keratinocyte homeostasis during the resolution phase of atopic dermatitis-like inflammation  
418 (Pellefigues, Naidoo, *et al.*, 2021). The differentiation of keratinocytes is critical for the quality of the  
419 wound healing response, as it restores the barrier function of the epidermis, which reduces microbial-  
420 driven local inflammation. Indeed, chronic wounds show hyperproliferative keratinocytes lacking the  
421 expression of terminal differentiation genes such as *Flg* and *Krt10*(Stojadinovic *et al.*, 2008;  
422 Wikramanayake, Stojadinovic and Tomic-Canic, 2014). Further studies will be necessary to determine  
423 if the control of keratinocyte homeostasis by basophils in skin wounds involves their direct secretion  
424 of IL-4, as suggested in a model of *S. aureus* infection(Strakosha *et al.*, 2024), or indirect mechanisms  
425 such as the control of the local inflammatory milieu. In agreement with the latter, we did not observe  
426 basophils close to the epidermis, and wound basophils seem biased to interact directly more with  
427 immune cells from the granulocyte–monocyte lineage than with keratinocytes (Figure 8G).

428 CSF1 is a critical regulator of monocyte/macrophage homeostasis through its interaction with the  
429 CSF1R. CSF1 is expressed constitutively at high levels by basophils (Uhlén *et al.*, 2015; Pellefigues,  
430 Naidoo, *et al.*, 2021) and many types of mesenchymal cells(Sehgal, Irvine and Hume, 2021). In the skin,  
431 CSF1 can promote monocyte/macrophage expansion and foster an M2-like bias in some particular  
432 models(Braza *et al.*, 2018; Pellefigues, Naidoo, *et al.*, 2021), as well as the resolution of inflammation  
433 during wound healing(Klinkert *et al.*, 2017; Kapanadze *et al.*, 2023). Similarly in our ear punch model,  
434 basophil-derived CSF1 accelerated wound closure during the first week and dampened the  
435 accumulation of Ly6C+ macrophages at 3 weeks post-wound. In the dorsal wound model, CSF1  
436 expression was increased in basophils from aged mice at day 4 post-wound. Overall, basophil-derived  
437 CSF1 seemed to influence the differentiation of wound macrophages, which was important to  
438 accelerate wound closure during the first week of healing. The specific deletion of basophils' IL-4  
439 induced an accumulation of Ly6C+ monocytes and a decrease of both subsets of macrophages analyzed  
440 decreasing at the same time their expression of some M2-like markers. IL-4 promotes macrophage  
441 proliferation *in situ*(Jenkins *et al.*, 2011, 2013) and a monocyte-to-macrophage phenotypic  
442 transition(Wong *et al.*, 1992; de Waal Malefyt *et al.*, 1993; Dang *et al.*, 2023; Finlay *et al.*, 2023). While  
443 testing these hypotheses was out of the scope of this study, we could identify a key role for basophil-  
444 derived IL-4 to promote macrophage homeostasis, to cause an M2-like bias during the resolution  
445 phase, and to accelerate wound closure at three weeks post-wound. IL-4 signaling is critical to limit  
446 age-induced inflammation at the organism and macrophage levels(Franceschi *et al.*, 2018; Zhou *et al.*,  
447 2024), and notably promotes a central immunosuppressive myelopoiesis program in a model of  
448 chronic inflammation(LaMarche *et al.*, 2024). In this light, basophil's IL-4 secretion may be a key natural  
449 regulator counterbalancing monocyte/macrophage-dependent chronic inflammation. Future studies

450 will be needed to decipher how basophil-derived IL-4 acts locally at the inflammatory site, or  
451 systemically by influencing myelopoiesis.

452 The phenotype of basophils from aged mice has only been scarcely studied. One seminal study found  
453 increased numbers of basophils in the spleens and bone marrow of C57BL/6 aged mice (van Beek *et al.*,  
454 2018). We confirm the peripheral basophilia in aged mice and that aged mice basophils exhibit a  
455 decreased expression of CD200R3, as it is observed upon basophil activation. In addition, we found  
456 aged mice basophils express more CD11b, CXCR4, and IgE in the blood, while FcεR1α, the high-affinity  
457 IgE receptor remains stable. This last finding seems counterintuitive as most FcεR1α receptors are  
458 thought to be occupied by IgE *in vivo*, and as IgE binding stabilizes the membrane expression of FcεR1α  
459 (Turner and Kinet, 1999). However, previous studies show that germ-free or antibiotics-treated  
460 mice show high serum IgE, peripheral basophilia, and increased basophil IgE binding without any  
461 noticeable increase in FcεR1α expression (Hill *et al.*, 2012). As age-associated microbiota can alter the  
462 phenotype of basophils (van Beek *et al.*, 2018), it may have increased the binding of IgE to basophils in  
463 a similar manner. Importantly, basophils showed potent anti-inflammatory, pro-resolving, and pro-  
464 healing properties in aged mice, while displaying a transcriptomic signature biased towards tissue  
465 remodeling and homeostasis (Figures 8-9). This is unexpected, as aging induces a chronic low-grade  
466 inflammation state arising from cellular senescence and a defect in the resolution (Franceschi *et al.*,  
467 2018). During wound healing, macrophages fail to adopt a pro-resolutive state and to perform  
468 efferocytosis, which facilitates the accumulation of neutrophils and the establishment of non-healing  
469 chronic wounds (De Maeyer *et al.*, 2020; Dube *et al.*, 2022). Aging is also characterized by a defect in  
470 type 2 cytokine signaling, while exogenous IL-4 restores age-induced macrophage defects, and a  
471 healthy lifespan, in mice (Zhou *et al.*, 2024). Thus, basophil secretion of IL-4 in aged mice appears as a  
472 mechanism arising to counterbalance age-associated inflammation. The fact that basophils express  
473 ALOX15 in the wounds of aged mice suggests basophils may further promote the resolution of  
474 inflammation through the production of SPMs (Basil and Levy, 2016). Old age is characterized by an  
475 increasing interindividual variability, and old mice exhibit a more variable wound healing rate than  
476 adult mice (Mahmoudi *et al.*, 2019). Our observations suggest that this variability may be due partly to  
477 basophil infiltration and that individuals defective in basophil pro-resolving properties would be more  
478 susceptible to the establishment of chronic wounds.

479 Overall, we identify basophils as pro-resolving cells regulating wound inflammation, particularly in the  
480 context of aging. Harnessing these properties may lead to new therapeutic strategies to prevent  
481 chronic wounds development in the elderly.

482

## 483 **Methods**

### 484 **Mice and treatments**

485 MCPT8<sup>DTR</sup> (Wada *et al.*, 2010), CTM8 (Tchen *et al.*, 2022), IL-4<sup>Loxp</sup> and CSF1<sup>Loxp</sup> mice (Shibata *et al.*, 2018;  
486 Pellefigues, Naidoo, *et al.*, 2021) were bred and maintained on a C57BL/6J background. C57BL/6J and  
487 Rosa-DTA mice were from The Jackson Laboratory through Charles River Laboratories or Envigo. “Baso-  
488 KO” mice are CTM8xRosa-DTA (Tchen *et al.*, 2022). Littermates were used to compare mice of the same  
489 sex and age in individual experiments. For experiments related to wound closure kinetics, we found  
490 sex-related significant differences and thus analyzed male and female kinetics independently. Sex bias  
491 is always described in the figure’s legend. MCPT8<sup>DTR</sup> mice were bred and maintained in specific  
492 pathogen-free conditions, or conventional conditions for experiments involving Cre-Loxp  
493 recombination, as described in the figure’s legend. Most of the study was conducted in accordance  
494 with the French and European guidelines and approved by the local ethics committee “Comité

495 d'éthique Paris Nord N°121" and the "Ministère de l'enseignement supérieur, de la recherche et de  
496 l'innovation" under the authorization number APAFIS#25642. Basoph8x4C13R(Pellefigues *et al.*, 2019)  
497 mice were bred on a pure C57BL/6J background in specific pathogen-free conditions at the Malaghan  
498 Institute of Medical Research Biomedical Research Unit. All experimental protocols on  
499 Basoph8x4C13R(Pellefigues *et al.*, 2019) mice were approved by the Victoria University of Wellington  
500 Animal Ethic Committee (Permit 24432). Each experiment was performed according to institutional  
501 guidelines. MCPT8<sup>DTR</sup> basophil depletion was induced by intraperitoneal injection of 1µg of diptheria  
502 toxin ("DT", unnicked from Sigma-Aldrich) at the specified times. Blood was harvested by retroorbital  
503 puncture on live animals or intracardiac puncture after euthanasia. "Aged" mice refer to animals  
504 between 75 weeks and 90 weeks old while "young" animals refer to 8 to 14 weeks old animals, unless  
505 specified otherwise (Figure 8). Animals were anesthetized using 3% Isoflurane in an anesthesia system  
506 (MiniHub, TemSega) for both wounding and blood harvesting on live animals. Euthanasia was always  
507 performed in a controlled CO2 chamber (TemSega). In the "Ear punch" model, a sterile punch biopsy  
508 of 2mm was used at the center of the ear to create a circular wound (Kai medical). In the "Ear Tag"  
509 model, we used a 2mm plier-style ear punch (Fisherbrand, Thermo Fischer) to generate a circular  
510 wound at the center of the ear.

### 511 **Tissue pathology and microscopy**

512 Skin Formalin-fixed paraffined embedded (FFPE) 4µm sections of the wounds were stained with  
513 Masson's trichrome to quantify epidermal thickness and collagen deposition using ImageJ v1.54h (Fiji,  
514 NIH). For whole-mount immunofluorescence, wholemount ear leaflets were stained with SYTO9  
515 (1/50000) for nuclear counterstaining in Superblock (both from Thermo Fischer) for tdTomato  
516 basophils quantification using the CT-M8 mice. Alternatively, ear leaflets of C57BL/6J were  
517 permeabilized 10' in 0.3% Triton X100 (Sigma) in PBS, rinsed in PBS, and then stained 1h in PBS 2% BSA  
518 + 10µg/mL 2.4G2 with anti-vimentin AF647 (Biolegend) and counterstained using 500ng/mL DAPI  
519 dilactate (Invitrogen). For neutrophils and monocytes-macrophages quantification, ear leaflets of  
520 C57BL/6J were permeabilized 10' in 0.3% Triton X100 (Sigma) in PBS, rinsed in PBS, and then stained  
521 for 1h in PBS 2% BSA + 10µg/mL 2.4G2, 100µg/mL mouse IgG and 100µg/mL rat IgG (Jackson  
522 Immunoresearch) in the presence of anti-Ly6G AF488 (1A8) and anti-CD68 AF647 (FA-11) or their  
523 isotypes (1/100 each, Biolegend), and counterstained using 500ng/mL DAPI dilactate (Invitrogen), all  
524 at room temperature. A maximum projection of 4 to 5 overlapping z-stacks and stitching of 9 fields of  
525 view centered around the wound are represented. Immunofluorescence of epidermal keratins was  
526 done on FFPE sections of MCPT8<sup>DTR</sup> mice after Sodium Citrate buffer (10mM Sodium Citrate, 0.05%  
527 Tween 20, pH 6.0) antigen retrieval using a steamer (30'), 2 washes in PBS 2', 1 wash in PBS 0.3%  
528 TritonX100 10', 2 washes in PBS 2', and then stained overnight at 4C in Superblock (Thermo Fischer)  
529 +5% rat serum and the following primary antibodies: rabbit IgG anti-filaggrin (Poly19058) and chicken  
530 IgY anti-keratin 14 (Poly9060) from Biolegend and guinea pig IgG anti-keratin 10 from Progen (GP-K10),  
531 each at 1/400. For secondary staining, samples are rinsed in PBS 0.05% Tween 20 twice 5', then stained  
532 in Superblock + 5% goat serum (Jackson Immunoresearch) 2h at room temperature using "AffiniPure"  
533 donkey F(ab')<sub>2</sub> anti-rabbit IgG AF488, anti-chicken IgY AF594 and F(ab')<sub>2</sub> anti-guinea pig IgG AF647, all  
534 at 1/500 from Jackson Immunoresearch. Samples were rinsed twice in PBS 0.05% Tween20 5', and once  
535 in 10mM CuSO<sub>4</sub>/50mM NH<sub>4</sub>Cl 10', and in deionized water before mounting in Shandon Immuno-Mount  
536 (Thermo Fischer). Fluorescence was then analyzed on a LSM 780 Airyscan confocal microscope (Zeiss),  
537 always at 20x in water immersion. Image analysis was done on ImageJ v1.54h (Fiji, NIH).

### 538 **Mouse sample handling and flow cytometry**

539 Immediately after death, cardiac puncture was done using a 25G needle, and a minimum of 700 µL of  
540 blood was withdrawn in a heparinized tube. The harvested blood cells were resuspended in 5 mL of

541 ACK lysing buffer (150 mM NH<sub>4</sub>Cl, 12 mM NaHCO<sub>3</sub>, 1 mM EDTA, pH 7.4) at room temperature for 3  
542 min, then further incubated for 5 min at 4 °C. Subsequently, 10 mL of PBS were added and the sample  
543 was centrifuged at 500G for 5 min. When red blood cells were still present, cells were further incubated  
544 in ACK lysing buffer for 5 min at 4 °C and the steps outlined above were repeated until red blood cells  
545 were lysed. The remaining white blood cells were resuspended in FACS buffer ("FB": PBS 1% BSA, 0.05%  
546 NaN<sub>3</sub>, 1 mM EDTA). Ear skin was harvested immediately after sacrifice, split into dorsal and dorsal  
547 parts using tweezers, and chopped with scissors in 1mL IMDM (Gibco). Ear skin was then digested for  
548 30' 37C in a shaking incubator at 150 rpm in 2mL of IMDM containing 1mg/mL Collagenase IV and  
549 200µg/mL DNase I (Sigma). Digestion was stopped by adding 1mM final EDTA (Gibco) and  
550 homogenizing samples at 4C. Samples were then filtered through a 70µm nylon mesh (Falcon).

551 Cells were then washed in PBS and resuspended in 96 well U-bottomed plates (Costar) before being  
552 stained for viability using 1/200 Ghost 510 (Tonbo) 15' 4 C. Then cells were washed in FB, and stained  
553 extracellularly in a blocking solution of FB containing 10µg/mL 2.4G2 (BioXcell) and 100µL/mL of mouse  
554 IgG, 100µg/mL rat IgG and 100µg/mL of Armenian Hamster IgG (all from Jackson Immunoresearch)  
555 with necessary fluorophore-labeled antibodies for 20' 4C in the dark (Supplementary Table 1). Then  
556 cells were washed twice in FB and fixed in PBS 1% PFA (Sigma-Aldrich) 10' 4C before being washed in  
557 FB, and stored in FB 4C overnight before analysis on a 3 laser Aurora spectral flow cytometer (Cytek,  
558 figure 1D, Supplementary Figure 2) or X20 Special Order 5-lasers Fortessa (BD) flow cytometer (other  
559 figures). Subsequent analysis was done using FlowJo 10 (BD). Mfi always represents geometrical mean  
560 intensity and is used to quantify fluorescence intensity. Alternatively, FSC-A and SSC-A are quantified  
561 as median values due to the linear nature of the variable. Results have been normalized as % of change  
562 from control to pool data from experiments performed after significant flow cytometer baseline  
563 changes.

564 Skin cytokines or growth factors were quantified using LegendPlex Mouse Cytokine release syndrome  
565 and Hematopoietic stem cell kits (Biolegend). Briefly, ear skin was snap-frozen and stored at -80C  
566 immediately after euthanasia. Then, tissue was thawed in Cell lysis buffer (Cell Signaling) containing  
567 1/100 Phenylmethanesulfonyl fluoride (Sigma) and minced before being homogenized using 5mm  
568 stainless steel beads using a Tissue Lyzer II (Qiagen). Protein content was quantified using a BCA test  
569 (Qiagen). Legendplex assay was done as per the manufacturer's instructions using a X20 Special Order  
570 5-laser Fortessa (BD) flow cytometer.

## 571 **Molecular biology**

572 Whole skin gene expression was quantified on ear skin that was snap-frozen immediately after  
573 euthanasia and stored at -80C. Then 30 mg of ear skin was used to extract mRNA using the RNeasy  
574 fibrous Tissue kit and the Tissue Lizer II, as per the manufacturer's protocol (Qiagen). cDNA were done  
575 using the High-quality RNA to cDNA kit (Thermo Fischer) and stored at -20C until quantitative qPCR  
576 was done using the PowerUp SYBR Green Master Mix 2x (Thermo Fisher) using a CFX96 real-time  
577 thermocycler (Bio-Rad), as per the manufacturer's protocol. The primers described in Supplementary  
578 Table 2 were used.

## 579 **Single-cell RNA-seq data analysis**

580 We reanalyzed our previously published scRNA-seq dataset (Vu et al., 2022) of dorsal wound cells from  
581 young (8 weeks old) and aged (88 weeks old) mice at 4 and 7 days post wounding. To identify the  
582 basophil cell cluster (MCPT8+ Kit-) and perform differential gene expression analysis of basophil cells  
583 between young and aged mice, we performed subclustering analysis of the previously assigned "mast  
584 cells" using the Seurat R package (version 4.4.0)(Stuart *et al.*, 2019). Alternatively, for visual  
585 representation, mast cells and basophils were identified by t-SNE clustering using Cytonaut

586 (<https://www.cytonaut-scipio.bio/>). To minimize the difference between different versions of  
587 Chromium Kits, we only used the cells from v3 runs. The top 10 principal components with a suitable  
588 resolution in `FindClusters` function were used for identifying the basophil cell cluster. `FindAllMarker`  
589 function with parameters `min.pct` being 0.25 and `logfc.threshold` being 0.1 was used to find  
590 differentially expressed genes. GO biological process enrichment analysis was performed using the  
591 clusterProfiler 4.0 R package(Wu *et al.*, 2021). Cell-cell communication analysis was performed as in  
592 our previous study using CellChat (version 2.0) (Jin *et al.*, 2021; Vu *et al.*, 2022; Jin, Plikus and Nie,  
593 2023). Briefly, the communication probability between two interacting cell groups was quantified  
594 based on the average expression values of a ligand by one cell group and that of a receptor by another  
595 cell group, as well as their cofactors. We computed the average expression of signaling molecules per  
596 cell group using 10% truncated mean. The significant interactions were inferred using permutation  
597 tests.

### 598 **Statistics and data visualization**

599 Statistical analyses were performed and plotted using Prism v9.x or v10.x (GraphPad). Data from  
600 experimental replicates were pooled if doing so led to a decreased variance; alternatively,  
601 representative results from an individual experiment were represented. Parametric or nonparametric  
602 tests were used depending on the normality of the data distribution assessed with a D'Agostino-  
603 Pearson K2 omnibus test. Posttest-adjusted p values are always represented when used. A two-tailed  
604 p-value less than 0.05 was considered the threshold for significance. Exact tests and post-tests are  
605 always described in the figure's legend.

### 606 **Data availability**

607 Data are available in the article itself and its supplementary materials. The scRNA-seq data was  
608 previously reported(Vu *et al.*, 2022), and is accessible in the GEO database under accession  
609 #GSE188432.

### 610 **Authors' contributions**

611 C.P. designed experiments, conducted experiments, acquired and analyzed data, wrote the  
612 manuscript, conceived and directed the project. N.C. and G.L.G. provided funding and edited the  
613 manuscript. J.B., V.P., C.S., L.C., O.T., M.K.T., J.T., Q.S., K.N., and G.G. conducted experiments, acquired  
614 data, managed animals, and/or edited the manuscript. S.J. analyzed bioinformatic data and/or edited  
615 the manuscript. X.D., K.M., H.K., J.X.J., M.B., R. M., and U.B. provided resources and edited the  
616 manuscript. C.P. has full access to all of the data in the study and takes responsibility for the integrity  
617 of the data and the accuracy of the data analysis. All authors approved the final version of the article.

### 618 **Funding**

619 This work was supported by the "Fondation pour la Recherche Médicale" (FRM) (grant #  
620 EQU201903007794) and the "Agence Nationale de la Recherche" (ANR) (grants # ANR-19-CE17-0029  
621 BALUMET) to NC and by an independent research organization grant from the Health Research Council  
622 of New Zealand and by the Marjorie Barclay Trust to G.L.G. and by the NIH (grant R01 AG045040) to  
623 J.X.J.. This research was also funded by the ANR grant ANRPIA-10-LABX-0017 INFLAMEX to the Centre  
624 de Recherche sur l'Inflammation, by the "Centre National de la Recherche Scientifique" (CNRS), by  
625 "Université de Paris", and by the "Institut National de la Santé et de la Recherche Médicale" (INSERM).

### 626 **Conflicts of interest**

627 The authors declare no conflict of interest with the results of the current study.



628 **Acknowledgments**

629 We acknowledge the expert work from the members of the UMR1149 “Centre de Recherche sur  
630 inflammation” animal (I. Renault and S. Olivré), flow cytometry (J. Da Silva, and V. Gratio), and imaging  
631 core facilities (S. Benadda); and the help from L. Wingertsmann from the morphology core facility  
632 (INSERM UMR1152). We also wish to thank the expert support of the Malaghan Institute of Medical  
633 Research Hugh Green Cytometry Core, Research Information Technologies, and Biomedical Research  
634 Unit staff.

635

636 **References**

637 Basavarajappa, D., Wan, M., Lukic, A., Steinhilber, D., Samuelsson, B. and Rådmark, O. (2014) ‘Roles  
638 of coactosin-like protein (CLP) and 5-lipoxygenase-activating protein (FLAP) in cellular leukotriene  
639 biosynthesis’, *Proceedings of the National Academy of Sciences*, 111(31), pp. 11371–11376. Available  
640 at: <https://doi.org/10.1073/pnas.1410983111>.

641 Basil, M.C. and Levy, B.D. (2016) ‘Specialized pro-resolving mediators: endogenous regulators of  
642 infection and inflammation. TL - 16’, *Nature reviews. Immunology*, 16 VN-r(1), pp. 51–67. Available  
643 at: <https://doi.org/10.1038/nri.2015.4>.

644 van Beek, A.A., Fransen, F., Meijer, B., de Vos, P., Knol, E.F. and Savelkoul, H.F.J. (2018) ‘Aged mice  
645 display altered numbers and phenotype of basophils, and bone marrow-derived basophil activation,  
646 with a limited role for aging-associated microbiota’, *Immunity & Ageing*, 15(1), p. 32. Available at:  
647 <https://doi.org/10.1186/s12979-018-0135-6>.

648 Blériot, C., Dupuis, T., Jouvion, G., Eberl, G., Disson, O. and Lecuit, M. (2015) ‘Liver-Resident  
649 Macrophage Necroptosis Orchestrates Type 1 Microbicidal Inflammation and Type-2-Mediated  
650 Tissue Repair during Bacterial Infection’, *Immunity*, 42(1), pp. 145–158. Available at:  
651 <https://doi.org/10.1016/j.immuni.2014.12.020>.

652 Bornes, L., Windoffer, R., Leube, R.E., Morgner, J. and van Rheenen, J. (2021) ‘Scratch-induced partial  
653 skin wounds re-epithelialize by sheets of independently migrating keratinocytes’, *Life Science  
654 Alliance*, 4(1), p. e202000765. Available at: <https://doi.org/10.26508/lsa.202000765>.

655 Braza, M.S., Conde, P., Garcia, M., Cortegano, I., Brahmachary, M., Pothula, V., Fay, F., Boros, P.,  
656 Werner, S.A., Ginhoux, F., Mulder, W.J.M. and Ochando, J. (2018) ‘Neutrophil derived CSF1 induces  
657 macrophage polarization and promotes transplantation tolerance’, *American Journal of  
658 Transplantation*, 18(5), pp. 1247–1255. Available at: <https://doi.org/10.1111/ajt.14645>.

659 Brigger, D., Riether, C., van Brummelen, R., Mosher, K.I., Shiu, A., Ding, Z., Zbären, N., Gasser, P.,  
660 Guntern, P., Yousef, H., Castellano, J.M., Storni, F., Graff-Radford, N., Britschgi, M., Grandgirard, D.,  
661 Hinterbrandner, M., Siegrist, M., Moullan, N., Hofstetter, W., Leib, S.L., Villiger, P.M., Auwerx, J.,  
662 Villeda, S.A., Wyss-Coray, T., Noti, M. and Eggel, A. (2020) ‘Eosinophils regulate adipose tissue  
663 inflammation and sustain physical and immunological fitness in old age’, *Nature Metabolism*, 2(8),  
664 pp. 688–702. Available at: <https://doi.org/10.1038/s42255-020-0228-3>.

665 Cohen, M., Giladi, A., Gorki, A.-D., Solodkin, D.G., Zada, M., Hladik, A., Miklosi, A., Salame, T.-M.,  
666 Halpern, K.B., David, E., Itzkovitz, S., Harkany, T., Knapp, S. and Amit, I. (2018) ‘Lung Single-Cell  
667 Signaling Interaction Map Reveals Basophil Role in Macrophage Imprinting’, *Cell*, 175(4), pp. 1031-  
668 1044.e18. Available at: <https://doi.org/10.1016/j.cell.2018.09.009>.

- 669 Cui, A., Huang, T., Li, S., Ma, A., Pérez, J.L., Sander, C., Keskin, D.B., Wu, C.J., Fraenkel, E. and  
670 Hacoheh, N. (2024) 'Dictionary of immune responses to cytokines at single-cell resolution', *Nature*,  
671 625(7994), pp. 377–384. Available at: <https://doi.org/10.1038/s41586-023-06816-9>.
- 672 Dang, B., Gao, Q., Zhang, L., Zhang, J., Cai, H., Zhu, Y., Zhong, Q., Liu, J., Niu, Y., Mao, K., Xiao, N., Liu,  
673 W.-H., Lin, S., Huang, J., Huang, S.C.-C., Ho, P.-C. and Cheng, S.-C. (2023) 'The glycolysis/HIF-1 $\alpha$  axis  
674 defines the inflammatory role of IL-4-primed macrophages', *Cell Reports*, 42(5), p. 112471. Available  
675 at: <https://doi.org/10.1016/j.celrep.2023.112471>.
- 676 De Maeyer, R.P.H., van de Merwe, R.C., Louie, R., Bracken, O.V., Devine, O.P., Goldstein, D.R., Uddin,  
677 M., Akbar, A.N. and Gilroy, D.W. (2020) 'Blocking elevated p38 MAPK restores efferocytosis and  
678 inflammatory resolution in the elderly', *Nature Immunology*, 21(6), pp. 615–625. Available at:  
679 <https://doi.org/10.1038/s41590-020-0646-0>.
- 680 Dube, C.T., Ong, Y.H.B., Wemyss, K., Krishnan, S., Tan, T.J., Janela, B., Grainger, J.R., Ronshaugen, M.,  
681 Mace, K.A. and Lim, C.Y. (2022) 'Age-Related Alterations in Macrophage Distribution and Function  
682 Are Associated With Delayed Cutaneous Wound Healing', *Frontiers in Immunology*, 13, p. 943159.  
683 Available at: <https://doi.org/10.3389/fimmu.2022.943159>.
- 684 Dubey, S.K., Dubey, R. and Kleinman, M.E. (2024) 'Unraveling Histone Loss in Aging and Senescence',  
685 *Cells*, 13(4), p. 320. Available at: <https://doi.org/10.3390/cells13040320>.
- 686 Egawa, M., Mukai, K., Yoshikawa, S., Iki, M., Mukaida, N., Kawano, Y., Minegishi, Y. and Karasuyama,  
687 H. (2013) 'Inflammatory Monocytes Recruited to Allergic Skin Acquire an Anti-inflammatory M2  
688 Phenotype via Basophil-Derived Interleukin-4', *Immunity*, 38(3), pp. 570–580. Available at:  
689 <https://doi.org/10.1016/j.immuni.2012.11.014>.
- 690 Ferrer-Font, L., Pellefigues, C., Mayer, J.U., Small, S.J., Jaimes, M.C. and Price, K.M. (2020) 'Panel  
691 Design and Optimization for High-Dimensional Immunophenotyping Assays Using Spectral Flow  
692 Cytometry', *Current Protocols in Cytometry*, 92(1). Available at: <https://doi.org/10.1002/cpcy.70>.
- 693 Ferrucci, L., Corsi, A., Lauretani, F., Bandinelli, S., Bartali, B., Taub, D.D., Guralnik, J.M. and Longo, D.L.  
694 (2005) 'The origins of age-related proinflammatory state', *Blood*, 105(6), pp. 2294–2299. Available at:  
695 <https://doi.org/10.1182/blood-2004-07-2599>.
- 696 Finlay, C.M., Parkinson, J.E., Zhang, L., Chan, B.H.K., Ajendra, J., Chenery, A., Morrison, A., Kaymak, I.,  
697 Houlder, E.L., Murtuza Baker, S., Dickie, B.R., Boon, L., Konkell, J.E., Hepworth, M.R., MacDonald, A.S.,  
698 Randolph, G.J., Rückerl, D. and Allen, J.E. (2023) 'T helper 2 cells control monocyte to tissue-resident  
699 macrophage differentiation during nematode infection of the pleural cavity', *Immunity*, 56(5), pp.  
700 1064-1081.e10. Available at: <https://doi.org/10.1016/j.immuni.2023.02.016>.
- 701 Franceschi, C., Garagnani, P., Parini, P., Giuliani, C. and Santoro, A. (2018) 'Inflammaging: a new  
702 immune–metabolic viewpoint for age-related diseases', *Nature Reviews Endocrinology*, 14(10), pp.  
703 576–590. Available at: <https://doi.org/10.1038/s41574-018-0059-4>.
- 704 Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy,  
705 D.W., Fasano, A., Miller, G.W., Miller, A.H., Mantovani, A., Weyand, C.M., Barzilai, N., Goronzy, J.J.,  
706 Rando, T.A., Effros, R.B., Lucia, A., Kleinstreuer, N. and Slavich, G.M. (2019) 'Chronic inflammation in  
707 the etiology of disease across the life span', *Nature Medicine*, 25(12), pp. 1822–1832. Available at:  
708 <https://doi.org/10.1038/s41591-019-0675-0>.

- 709 Gause, W.C., Wynn, T.A. and Allen, J.E. (2013) 'Type 2 immunity and wound healing: evolutionary  
710 refinement of adaptive immunity by helminths', *Nature Reviews Immunology*, 13(8), pp. 607–614.  
711 Available at: <https://doi.org/10.1038/nri3476>.
- 712 Gieseck, R.L., Wilson, M.S. and Wynn, T.A. (2018) 'Type 2 immunity in tissue repair and fibrosis',  
713 *Nature reviews. Immunology*, 18(1), pp. 62–76. Available at: <https://doi.org/10.1038/nri.2017.90>.
- 714 Hachem, C.E., Marschall, P., Hener, P., Karnam, A., Bonam, S.R., Meyer, P., Flatter, E., Birling, M.-C.,  
715 Bayry, J. and Li, M. (2023) 'IL-3 produced by T cells is crucial for basophil extravasation in hapten-  
716 induced allergic contact dermatitis', *Frontiers in Immunology*, 14, p. 1151468. Available at:  
717 <https://doi.org/10.3389/fimmu.2023.1151468>.
- 718 Hayes, M.D., Ward, S., Crawford, G., Seoane, R.C., Jackson, W.D., Kipling, D., Voehringer, D., Dunn-  
719 Walters, D. and Strid, J. (2020) 'Inflammation-induced IgE promotes epithelial hyperplasia and  
720 tumour growth', *eLife*, 9. Available at: <https://doi.org/10.7554/eLife.51862>.
- 721 Hill, D.A., Siracusa, M.C., Abt, M.C., Kim, B.S., Kobuley, D., Kubo, M., Kambayashi, T., LaRosa, D.F.,  
722 Renner, E.D., Orange, J.S., Bushman, F.D. and Artis, D. (2012) 'Commensal bacteria-derived signals  
723 regulate basophil hematopoiesis and allergic inflammation', *Nature Medicine*, 18(4), pp. 538–546.  
724 Available at: <https://doi.org/10.1038/nm.2657>.
- 725 Iwamoto, H., Matsubara, T., Nakazato, Y., Namba, K. and Takeda, Y. (2015) 'Decreased expression of  
726 CD200R3 on mouse basophils as a novel marker for IgG1-mediated anaphylaxis', *Immunity,  
727 Inflammation and Disease*, 3(3), pp. 280–288. Available at: <https://doi.org/10.1002/iid3.67>.
- 728 Jenkins, S.J., Ruckerl, D., Cook, P.C., Jones, L.H., Finkelman, F.D., van Rooijen, N., MacDonald, A.S. and  
729 Allen, J.E. (2011) 'Local Macrophage Proliferation, Rather than Recruitment from the Blood, Is a  
730 Signature of TH2 Inflammation', *Science*, 332(6035), pp. 1284–1288. Available at:  
731 <https://doi.org/10.1126/science.1204351>.
- 732 Jenkins, S.J., Ruckerl, D., Thomas, G.D., Hewitson, J.P., Duncan, S., Brombacher, F., Maizels, R.M.,  
733 Hume, D.A. and Allen, J.E. (2013) 'IL-4 directly signals tissue-resident macrophages to proliferate  
734 beyond homeostatic levels controlled by CSF-1', *The Journal of Experimental Medicine*, 210(11), pp.  
735 2477–2491. Available at: <https://doi.org/10.1084/jem.20121999>.
- 736 Jin, S., Guerrero-Juarez, C.F., Zhang, L., Chang, I., Ramos, R., Kuan, C.-H., Myung, P., Plikus, M.V. and  
737 Nie, Q. (2021) 'Inference and analysis of cell-cell communication using CellChat', *Nature  
738 Communications*, 12(1), p. 1088. Available at: <https://doi.org/10.1038/s41467-021-21246-9>.
- 739 Jin, S., Plikus, M.V. and Nie, Q. (2023) 'CellChat for systematic analysis of cell-cell communication  
740 from single-cell and spatially resolved transcriptomics'. Biorxiv [Preprint]. Available at:  
741 <https://doi.org/10.1101/2023.11.05.565674>.
- 742 Kapanadze, T., Gamrekelashvili, J., Sablotny, S., Kijas, D., Haller, H., Schmidt-Ott, K. and Limbourg, F.P.  
743 (2023) 'CSF-1 and Notch signaling cooperate in macrophage instruction and tissue repair during  
744 peripheral limb ischemia', *Frontiers in Immunology*, 14, p. 1240327. Available at:  
745 <https://doi.org/10.3389/fimmu.2023.1240327>.
- 746 Kleiner, S., Rüdrieh, U., Gehring, M., Loser, K., Eiz-Vesper, B., Noubissi Nzeteu, G.A., Patsinakidis, N.,  
747 Meyer, N.H., Gibbs, B.F. and Raap, U. (2021) 'Human basophils release the anti-inflammatory  
748 cytokine IL-10 following stimulation with  $\alpha$ -melanocyte-stimulating hormone', *Journal of Allergy and  
749 Clinical Immunology*, p. S0091674921000099. Available at:  
750 <https://doi.org/10.1016/j.jaci.2020.12.645>.

- 751 Klinkert, K., Whelan, D., Clover, A.J.P., Leblond, A.-L., Kumar, A.H.S. and Caplice, N.M. (2017)  
752 'Selective M2 Macrophage Depletion Leads to Prolonged Inflammation in Surgical Wounds',  
753 *European Surgical Research*, 58(3–4), pp. 109–120. Available at: <https://doi.org/10.1159/000451078>.
- 754 Knoops, B., Goemaere, J., Van Der Eecken, V. and Declercq, J.-P. (2011) 'Peroxiredoxin 5: Structure,  
755 Mechanism, and Function of the Mammalian Atypical 2-Cys Peroxiredoxin', *Antioxidants & Redox*  
756 *Signaling*, 15(3), pp. 817–829. Available at: <https://doi.org/10.1089/ars.2010.3584>.
- 757 Konieczny, P., Xing, Y., Sidhu, I., Subudhi, I., Mansfield, K.P., Hsieh, B., Biancur, D.E., Larsen, S.B.,  
758 Cammer, M., Li, D., Landén, N.X., Loomis, C., Heguy, A., Tikhonova, A.N., Tsigirgos, A. and Naik, S.  
759 (2022) 'Interleukin-17 governs hypoxic adaptation of injured epithelium', *Science*, p. eabg9302.  
760 Available at: <https://doi.org/10.1126/science.abg9302>.
- 761 Krzyszczyk, P., Schloss, R., Palmer, A. and Berthiaume, F. (2018) 'The Role of Macrophages in Acute  
762 and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes', *Frontiers*  
763 *in Physiology*, 9(MAY), p. 419. Available at: <https://doi.org/10.3389/fphys.2018.00419>.
- 764 LaMarche, N.M., Hegde, S., Park, M.D., Maier, B.B., Troncoso, L., Le Berichel, J., Hamon, P., Belabed,  
765 M., Mattiuz, R., Hennequin, C., Chin, T., Reid, A.M., Reyes-Torres, I., Nemeth, E., Zhang, R., Olson,  
766 O.C., Doroshov, D.B., Rohs, N.C., Gomez, J.E., Veluswamy, R., Hall, N., Venturini, N., Ginhoux, F., Liu,  
767 Z., Buckup, M., Figueiredo, I., Roudko, V., Miyake, K., Karasuyama, H., Gonzalez-Kozlova, E., Gnjatic,  
768 S., Passegué, E., Kim-Schulze, S., Brown, B.D., Hirsch, F.R., Kim, B.S., Marron, T.U. and Merad, M.  
769 (2024) 'An IL-4 signalling axis in bone marrow drives pro-tumorigenic myelopoiesis', *Nature*,  
770 625(7993), pp. 166–174. Available at: <https://doi.org/10.1038/s41586-023-06797-9>.
- 771 Leyva-Castillo, J.-M., Das, M., Kane, J., Strakosha, M., Singh, S., Wong, D.S.H., Horswill, A.R.,  
772 Karasuyama, H., Brombacher, F., Miller, L.S. and Geha, R.S. (2021) 'Basophil-derived IL-4 promotes  
773 cutaneous Staphylococcus aureus infection', *JCI Insight*, 6(21), p. e149953. Available at:  
774 <https://doi.org/10.1172/jci.insight.149953>.
- 775 Leyva-Castillo, J.M., Sun, L., Wu, S.-Y., Rockowitz, S., Sliz, P. and Geha, R. (2022) 'Single cell  
776 transcriptome profile of mouse skin undergoing antigen driven allergic inflammation recapitulates  
777 findings in atopic dermatitis skin lesions', *Journal of Allergy and Clinical Immunology*, p.  
778 S0091674922002998. Available at: <https://doi.org/10.1016/j.jaci.2022.03.002>.
- 779 Mahmoudi, S., Mancini, E., Xu, L., Moore, A., Jahanbani, F., Hebestreit, K., Srinivasan, R., Li, X.,  
780 Devarajan, K., Prélôt, L., Ang, C.E., Shibuya, Y., Benayoun, B.A., Chang, A.L.S., Wernig, M., Wysocka, J.,  
781 Longaker, M.T., Snyder, M.P. and Brunet, A. (2019) 'Heterogeneity in old fibroblasts is linked to  
782 variability in reprogramming and wound healing', *Nature*, 574(7779), pp. 553–558. Available at:  
783 <https://doi.org/10.1038/s41586-019-1658-5>.
- 784 Miyake, K., Ito, J. and Karasuyama, H. (2022) 'Role of Basophils in a Broad Spectrum of Disorders',  
785 *Frontiers in Immunology*, 13, p. 902494. Available at: <https://doi.org/10.3389/fimmu.2022.902494>.
- 786 Miyake, K., Ito, J., Takahashi, K., Nakabayashi, J., Brombacher, F., Shichino, S., Yoshikawa, S., Miyake,  
787 S. and Karasuyama, H. (2024) 'Single-cell transcriptomics identifies the differentiation trajectory from  
788 inflammatory monocytes to pro-resolving macrophages in a mouse skin allergy model', *Nature*  
789 *Communications*, 15(1), p. 1666. Available at: <https://doi.org/10.1038/s41467-024-46148-4>.
- 790 Nascimento-Filho, C.H.V., Silveira, E.J.D., Goloni-Bertollo, E.M., de Souza, L.B., Squarize, C.H. and  
791 Castilho, R.M. (2020) 'Skin wound healing triggers epigenetic modifications of histone H4', *Journal of*  
792 *Translational Medicine*, 18(1), p. 138. Available at: <https://doi.org/10.1186/s12967-020-02303-1>.

- 793 Nishiguchi, M.A., Spencer, C.A., Leung, D.H. and Leung, T.H. (2018) 'Aging Suppresses Skin-Derived  
794 Circulating SDF1 to Promote Full-Thickness Tissue Regeneration', *Cell Reports*, 24(13), pp. 3383-  
795 3392.e5. Available at: <https://doi.org/10.1016/j.celrep.2018.08.054>.
- 796 Nishikoba, N., Kumagai, K., Kanmura, S., Nakamura, Y., Ono, M., Eguchi, H., Kamibayashiyama, T.,  
797 Oda, K., Mawatari, S., Tanoue, S., Hashimoto, S., Tsubouchi, H. and Ido, A. (2020) 'HGF-MET Signaling  
798 Shifts M1 Macrophages Toward an M2-Like Phenotype Through PI3K-Mediated Induction of  
799 Arginase-1 Expression', *Frontiers in Immunology*, 11, p. 2135. Available at:  
800 <https://doi.org/10.3389/fimmu.2020.02135>.
- 801 van Panhuys, N., Prout, M., Forbes, E., Min, B., Paul, W.E. and Le Gros, G. (2011) 'Basophils are the  
802 major producers of IL-4 during primary helminth infection.', *Journal of immunology (Baltimore, Md. :  
803 1950)*, 186(5), pp. 2719–2728. Available at: <https://doi.org/10.4049/jimmunol.1000940>.
- 804 Pellefigues, C., Dema, B., Lamri, Y., Saidoune, F., Chavarot, N., Lohéac, C., Pacreau, E., Dussiot, M.,  
805 Bidault, C., Marquet, F., Jablonski, M., Chemouny, J.M., Jouan, F., Dossier, A., Chauveheid, M.-P.,  
806 Gobert, D., Papo, T., Karasuyama, H., Sacré, K., Daugas, E. and Charles, N. (2018) 'Prostaglandin D2  
807 amplifies lupus disease through basophil accumulation in lymphoid organs', *Nature Communications*,  
808 9(1), p. 725. Available at: <https://doi.org/10.1038/s41467-018-03129-8>.
- 809 Pellefigues, C., Mehta, P., Chappell, S., Yumnam, B., Old, S., Camberis, M. and Le Gros, G. (2021)  
810 'Diverse innate stimuli activate basophils through pathways involving Syk and I $\kappa$ B kinases',  
811 *Proceedings of the National Academy of Sciences*, 118(12), p. e2019524118. Available at:  
812 <https://doi.org/10.1073/pnas.2019524118>.
- 813 Pellefigues, C., Mehta, P., Prout, M.S., Naidoo, K., Yumnam, B., Chandler, J., Chappell, S., Filbey, K.,  
814 Camberis, M. and Le Gros, G. (2019) 'The Basoph8 Mice Enable an Unbiased Detection and a  
815 Conditional Depletion of Basophils', *Frontiers in Immunology*, 10, p. 2143. Available at:  
816 <https://doi.org/10.3389/fimmu.2019.02143>.
- 817 Pellefigues, C., Naidoo, K., Mehta, P., Schmidt, A.J., Jagot, F., Roussel, E., Cait, A., Yumnam, B.,  
818 Chappell, S., Meijlink, K., Camberis, M., Jiang, J.X., Painter, G., Filbey, K., Uluçkan, Ö., Gasser, O. and  
819 Le Gros, G. (2021) 'Basophils promote barrier dysfunction and resolution in the atopic skin', *Journal  
820 of Allergy and Clinical Immunology*, p. S0091674921002815. Available at:  
821 <https://doi.org/10.1016/j.jaci.2021.02.018>.
- 822 Philp, D. and Kleinman, H.K. (2010) 'Animal studies with thymosin  $\beta_4$ , a multifunctional tissue repair  
823 and regeneration peptide', *Annals of the New York Academy of Sciences*, 1194(1), pp. 81–86.  
824 Available at: <https://doi.org/10.1111/j.1749-6632.2010.05479.x>.
- 825 Poto, R., Loffredo, S., Marone, G., Di Salvatore, A., De Paulis, A., Schroeder, J.T. and Varricchi, G.  
826 (2023) 'Basophils beyond allergic and parasitic diseases', *Frontiers in Immunology*, 14, p. 1190034.  
827 Available at: <https://doi.org/10.3389/fimmu.2023.1190034>.
- 828 Rajnoch, C., Ferguson, S., Metcalfe, A.D., Herrick, S.E., Willis, H.S. and Ferguson, M.W.J. (2003)  
829 'Regeneration of the ear after wounding in different mouse strains is dependent on the severity of  
830 wound trauma', *Developmental Dynamics*, 226(2), pp. 388–397. Available at:  
831 <https://doi.org/10.1002/dvdy.10242>.
- 832 Rutella, S. (2006) 'Hepatocyte growth factor favors monocyte differentiation into regulatory  
833 interleukin (IL)-10+IL-12low/neg accessory cells with dendritic-cell features', *Blood*, 108(1), pp. 218–  
834 227. Available at: <https://doi.org/10.1182/blood-2005-08-3141>.

- 835 Sehgal, A., Irvine, K.M. and Hume, D.A. (2021) 'Functions of macrophage colony-stimulating factor  
836 (CSF1) in development, homeostasis, and tissue repair', *Seminars in Immunology*, 54, p. 101509.  
837 Available at: <https://doi.org/10.1016/j.smim.2021.101509>.
- 838 Serhan, C.N. (2014) 'Pro-resolving lipid mediators are leads for resolution physiology.', *Nature*,  
839 510(7503), pp. 92–101. Available at: <https://doi.org/10.1038/nature13479>.
- 840 Shibata, S., Miyake, K., Tateishi, T., Yoshikawa, S., Yamanishi, Y., Miyazaki, Y., Inase, N. and  
841 Karasuyama, H. (2018) 'Basophils trigger emphysema development in a murine model of COPD  
842 through IL-4-mediated generation of MMP-12-producing macrophages.', *Proceedings of the National  
843 Academy of Sciences of the United States of America*, 115(51), pp. 13057–13062. Available at:  
844 <https://doi.org/10.1073/pnas.1813927115>.
- 845 Sicklinger, F., Meyer, I.S., Li, X., Radtke, D., Dicks, S., Kornadt, M.P., Mertens, C., Meier, J.K., Lavine,  
846 K.J., Zhang, Y., Kuhn, T.C., Terzer, T., Patel, J., Boerries, M., Schramm, G., Frey, N., Katus, H.A.,  
847 Voehringer, D. and Leuschner, F. (2021) 'Basophils balance healing after myocardial infarction via IL-  
848 4/IL-13', *Journal of Clinical Investigation*, 131(13), p. e136778. Available at:  
849 <https://doi.org/10.1172/JCI136778>.
- 850 Stojadinovic, O., Pastar, I., Vukelic, S., Mahoney, M.G., Brennan, D., Krzyzanowska, A., Golinko, M.,  
851 Brem, H. and Tomic-Canic, M. (2008) 'Deregulation of keratinocyte differentiation and activation: a  
852 hallmark of venous ulcers', *Journal of Cellular and Molecular Medicine*, 12(6b), pp. 2675–2690.  
853 Available at: <https://doi.org/10.1111/j.1582-4934.2008.00321.x>.
- 854 Strakosha, M., Vega-Mendoza, D., Kane, J., Jain, A., Sun, L., Rockowitz, S., Elkins, M., Miyake, K.,  
855 Chou, J., Karasuyama, H., Geha, R.S. and Leyva-Castillo, J.-M. (2024) 'Basophils play a protective role  
856 in the recovery of skin barrier function from mechanical injury in mice.', *Journal of Investigative  
857 Dermatology*, p. S0022202X24000794. Available at: <https://doi.org/10.1016/j.jid.2023.12.024>.
- 858 Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W.M., Hao, Y., Stoeckius, M.,  
859 Smibert, P. and Satija, R. (2019) 'Comprehensive Integration of Single-Cell Data', *Cell*, 177(7), pp.  
860 1888-1902.e21. Available at: <https://doi.org/10.1016/j.cell.2019.05.031>.
- 861 Takahashi, K., Miyake, K., Ito, J., Shimamura, H., Suenaga, T., Karasuyama, H. and Ohashi, K. (2023)  
862 'Topical Application of a PDE4 Inhibitor Ameliorates Atopic Dermatitis through Inhibition of Basophil  
863 IL-4 Production', *Journal of Investigative Dermatology*, p. S0022202X23029202. Available at:  
864 <https://doi.org/10.1016/j.jid.2023.09.272>.
- 865 Tchen, J., Simon, Q., Chapart, L., Pellefigues, C., Karasuyama, H., Miyake, K., Blank, U., Benhamou, M.,  
866 Daugas, E. and Charles, N. (2022) 'CT-M8 Mice: A New Mouse Model Demonstrates That Basophils  
867 Have a Nonredundant Role in Lupus-Like Disease Development', *Frontiers in Immunology*, 13, p.  
868 900532. Available at: <https://doi.org/10.3389/fimmu.2022.900532>.
- 869 Tsai, S.H., Kinoshita, M., Kusu, T., Kayama, H., Okumura, R., Ikeda, K., Shimada, Y., Takeda, A.,  
870 Yoshikawa, S., Obata-Ninomiya, K., Kurashima, Y., Sato, S., Umemoto, E., Kiyono, H., Karasuyama, H.  
871 and Takeda, K. (2015) 'The Ectoenzyme E-NPP3 Negatively Regulates ATP-Dependent Chronic Allergic  
872 Responses by Basophils and Mast Cells', *Immunity*, 42(2), pp. 279–293. Available at:  
873 <https://doi.org/10.1016/j.immuni.2015.01.015>.
- 874 Turner, H. and Kinet, J.-P. (1999) 'Signalling through the high-affinity IgE receptor FcεRI', *Nature*,  
875 402(S6760), pp. 24–30. Available at: <https://doi.org/10.1038/35037021>.

- 876 Uhlén, M., Fagerberg, L., Hallström, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å.,  
877 Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigartyo, C.A.-K.,  
878 Odeberg, J., Djureinovic, D., Takanen, J.O., Hober, S., Alm, T., Edqvist, P.-H., Berling, H., Tegel, H.,  
879 Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., Von Feilitzen, K., Forsberg, M.,  
880 Persson, L., Johansson, F., Zwahlen, M., Von Heijne, G., Nielsen, J. and Pontén, F. (2015) 'Tissue-based  
881 map of the human proteome', *Science*, 347(6220), p. 1260419. Available at:  
882 <https://doi.org/10.1126/science.1260419>.
- 883 Vivanco Gonzalez, N., Oliveria, J.-P., Tebaykin, D., Ivison, G.T., Mukai, K., Tsai, M.M., Borges, L.,  
884 Nadeau, K.C., Galli, S.J., Tsai, A.G. and Bendall, S.C. (2020) 'Mass Cytometry Phenotyping of Human  
885 Granulocytes Reveals Novel Basophil Functional Heterogeneity', *iScience*, 23(11), p. 101724. Available  
886 at: <https://doi.org/10.1016/j.isci.2020.101724>.
- 887 Vu, R., Jin, S., Sun, P., Haensel, D., Nguyen, Q.H., Dragan, M., Kessenbrock, K., Nie, Q. and Dai, X.  
888 (2022) 'Wound healing in aged skin exhibits systems-level alterations in cellular composition and cell-  
889 cell communication', *Cell Reports*, 40(5), p. 111155. Available at:  
890 <https://doi.org/10.1016/j.celrep.2022.111155>.
- 891 de Waal Malefyt, R., Figdor, C.G., Huijbens, R., Mohan-Peterson, S., Bennett, B., Culpepper, J., Dang,  
892 W., Zurawski, G. and de Vries, J.E. (1993) 'Effects of IL-13 on phenotype, cytokine production, and  
893 cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-  
894 10', *Journal of Immunology (Baltimore, Md.: 1950)*, 151(11), pp. 6370–6381.
- 895 Wada, T., Ishiwata, K., Koseki, H., Ishikura, T., Ugajin, T., Ohnuma, N., Obata, K., Ishikawa, R.,  
896 Yoshikawa, S., Mukai, K., Kawano, Y., Minegishi, Y., Yokozeki, H., Watanabe, N. and Karasuyama, H.  
897 (2010) 'Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity  
898 against ticks.', *The Journal of clinical investigation*, 120(8), pp. 2867–75. Available at:  
899 <https://doi.org/10.1172/JCI42680>.
- 900 Wikramanayake, T.C., Stojadinovic, O. and Tomic-Canic, M. (2014) 'Epidermal Differentiation in  
901 Barrier Maintenance and Wound Healing', *Advances in Wound Care*, 3(3), pp. 272–280. Available at:  
902 <https://doi.org/10.1089/wound.2013.0503>.
- 903 Wong, H.L., Lotze, M.T., Wahl, L.M. and Wahl, S.M. (1992) 'Administration of recombinant IL-4 to  
904 humans regulates gene expression, phenotype, and function in circulating monocytes', *Journal of*  
905 *Immunology (Baltimore, Md.: 1950)*, 148(7), pp. 2118–2125.
- 906 Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo,  
907 X. and Yu, G. (2021) 'clusterProfiler 4.0: A universal enrichment tool for interpreting omics data',  
908 *Innovation (Cambridge (Mass.))*, 2(3), p. 100141. Available at:  
909 <https://doi.org/10.1016/j.xinn.2021.100141>.
- 910 Zhou, Z., Yao, J., Wu, D., Huang, X., Wang, Y., Li, X., Lu, Q. and Qiu, Y. (2024) 'Type 2 cytokine signaling  
911 in macrophages protects from cellular senescence and organismal aging', *Immunity*, p.  
912 S1074761324000268. Available at: <https://doi.org/10.1016/j.immuni.2024.01.001>.

913

914

915

916 **Legends**

917 **Figure 1: Basophils are enriched and activated during the resolution of skin wound healing.** A-C) Wild-  
918 type mice were wounded by a sterile 2mm punch biopsy of the ear at day 0. A) Representative gating  
919 strategy of skin leukocytes among Living CD45+ Singlets and B) their proportions over time (n=23-40).  
920 C) Quantification of cytokines and chemokines by multiplex immunoassay over time, normalized by  
921 ear skin protein content (n=4-5). D) The phenotype of skin CD45+ CD64+ CD11b+ macrophages and  
922 CD45+ YFP+ basophils was analyzed by flow cytometry overtime after a 2mm plier-style ear punch in  
923 Basoph8x4C13R mice (n=4) as previously described (Ferrer-Font *et al.*, 2020). A 3rd order polynomial  
924 interpolation with a 95% confidence interval is represented. Results are pooled from 3 to 10  
925 independent experiments (B, C) or from a unique experiment (D). The inflammation and resolution  
926 phases are represented in red or blue with a day 7 cutoff. Statistics are ordinary one-way ANOVA  
927 corrected by a Holm-Sidak's multiple comparison test against day 0. p<0.05: \*; p<0.01: \*\*; p<0.001:  
928 \*\*\*; p<0.0001: \*\*\*\*

929

930 **Figure 2: Basophils infiltrate ear skin wounds during inflammation and resolution.** A) Representative  
931 photography of the area of the wound on day 1 and day 21 after ear punch biopsy (red dots). B-F) The  
932 ear skin was analyzed by whole mount confocal imaging of one dermal leaflet at (B-D) day 1, E) day 7,  
933 or F) day 21 after ear punch. B) The tdTomato fluorescence of a CTM8 mouse after counterstaining  
934 with SYTO9 without fixation or permeabilization and C) the expression of Ly6G, CD68, and CD31 of a  
935 wild-type mouse ear after gentle fixation and permeabilization and counterstaining with DAPI were  
936 used D) to quantify the closest distance of each of these cell types to the wound edge (n=360, 300,  
937 559). E, F) The tdTomato fluorescence of CTM8 mice was used to identify basophils in the ear wounds  
938 at different time points under conditions of gentle fixation/permeabilization, alongside vimentin and  
939 DAPI counterstainings. B, C, E, F) Scale bars: 200µm. Each representation is a stitching of 9 images of  
940 the maximum projection of 3 to 5 overlapping z-stacks, at 10X. D) Results are pooled from three  
941 independent experiments. Statistics are a Kruskal-Wallis test corrected by a Dunn's multiple  
942 comparison test against MCPT8+ cells. p<0.0001: \*\*\*\*

943

944 **Figure 3: Basophils dampen activation of pro-inflammatory cells and inflammation during the first**  
945 **week of wound healing.** MCPT8-DTR mice were depleted specifically of basophils by diphtheria toxin  
946 injections A-C) at days -2, -1, and D-G) on day 5 post-wound. The infiltration of leukocytes, their  
947 proportions, and their phenotype were analyzed by flow cytometry A, B) at day 1 (n=16-24) or D, E) at  
948 day 7 (n=13-14). B, E) Each phenotype has been normalized on the control group (PBS) of each  
949 individual experiment for each population before pooling. Similarly, ear skin cytokine or chemokine  
950 content was quantified by multiplex immunoassay at C) day 1 or F) day 7 post wound, after  
951 normalization on total skin proteins (n=4-5). G) Skin gene expression was analyzed at day 0 and day 7  
952 after normalization on GAPDH expression (n=3-5). Results are pooled from three independent  
953 experiments. Statistics are two-tailed Mann-Whitney tests. ns: non significant; p>0.1: #; p<0.01: #;  
954 p<0.05: \*; p<0.01: \*\*; p<0.001: \*\*\*; p<0.0001: \*\*\*\*

955

956 **Figure 4: Basophils accelerate wound healing and the quality of the reepithelialization.** Wild-type  
957 (WT) female mice with a C57BL/6J genetic background were bred in a specific pathogen-free (SPF,



958 MCPT8<sup>DTR</sup> mice) or conventional animal facility (AF, CTM8 mice), were ear punched at D0, and wound  
959 closure was monitored overtime until D19 (n=14-16). Similarly, wound closure at D7 and D11 was  
960 compared between B) CTM8 and CTM8xRosa-DTA ("Baso-KO") mice bred in conventional AF (n=6-8)  
961 or C) MCPT8<sup>DTR</sup> mice bred in SPF conditions depleted of basophils by injections of diphteria toxin (DT)  
962 at Day -2, -1, and Day 5 (n=6), or not depleted after injections of PBS (n=12). D) The thickness of the  
963 epidermis directly adjacent to the wound and the length of the migrating epithelial tongue were  
964 measured on Masson's Trichrome colored ear sections at D1 post-wound in MCPT8<sup>DTR</sup> mice treated  
965 with DT or PBS (n=36-40 wound edges, 2/ear). A D1 representative Masson's trichrome coloration and  
966 a serial section stained with anti-filaggrin (FLG), Keratin 14 (K14), and Keratin 10 (K10) antibodies are  
967 depicted. AF (Blue) represents autofluorescence at 450nm. E) Similarly, the maximum wound bed  
968 epidermal thickness was measured over time and compared to D0 (n=16-32 wound edges, 2/ear) or F)  
969 measured at Day 7 in MCPT8<sup>DTR</sup> mice treated with DT or PBS. G) Representative immunofluorescent  
970 staining of ear wound sections at Day 7, as in D, allowed the quantification of K14+ keratinocytes  
971 (stratum basale), K10+ keratinocytes (stratum spinosum), and FLG+ keratinocytes (stratum  
972 granulosum), as depicted. H) Analysis of the proportions of various keratinocytes in the wound bed  
973 and the adjacent epidermis at Day 7 as in G, in mice as in C). F, H) (n=20 wound edges, 2/ear). A, B, C,  
974 H) Means +/- sem or D, E, F) Medians +/- interquartile range are represented. Statistics are A) Repeated  
975 measure ANOVA or B, D) two-way ANOVAs with Holm-Sidak's correction, or D, F) Mann Whitney tests  
976 or E) Kruskal-Wallis tests with Dunn's correction or H) unpaired t-tests comparing the PBS to DT  
977 condition for each subset. ns: non significant; p<0.05: \*; p<0.01: \*\*; p<0.001: \*\*\*; p<0.0001: \*\*\*\*

978

979 **Figure 5: Basophils dampen the accumulation and activation of pro-inflammatory leukocytes during**  
980 **the resolution phase of skin wound healing.** A) Wild type (WT) or basophil-deficient (Baso-KO)  
981 littermates were ear-punched and skin leukocytes were analyzed by flow cytometry at D21 (n=13, 8).  
982 B) Similarly, MCPT8<sup>DTR</sup> mice were ear punched and injected with diphteria toxin (DT) or PBS on Days  
983 13, 14, and 19 before being sacrificed on Day 21. C) The proportion of their skin leukocytes and D) their  
984 phenotype (normalized on control) were quantified by flow cytometry (n=14, 10). E) Similarly the  
985 chemokine CCL2 was quantified by immunoassay after normalization on total skin proteins (n=5) and  
986 F) the expression of CD206 and CD301b was quantified on Ly6C- macrophages by flow cytometry  
987 (n=14-10). Results are pooled from two to three independent experiments. Statistics are two-tailed  
988 Mann-Whitney tests. ns: non significant; p<0.05: \*; p<0.01: \*\*; p<0.001: \*\*\*; p<0.0001: \*\*\*\*

989

990 **Figure 6: Basophils CSF1 and IL-4 independently promote wound closure and show complex**  
991 **immunoregulatory properties.** Wild-type (WT) mice and mice with a basophil-specific deletion in Csf1  
992 (CTM8<sup>ΔCSF1</sup>) or Il4 (CTM8<sup>ΔIL4</sup>) expression were ear-punched at Day 0. A) Ear hole area was monitored  
993 overtime after normalization on day 0 (n= 19, 21, 10), and B) total leukocyte content was determined  
994 by flow cytometry on Day 21 (n=14, 16, 10). The proportion and phenotype of skin leukocytes were  
995 analyzed by flow cytometry for C, D) CTM8<sup>ΔCSF1</sup> or E, F, G) CTM8<sup>ΔIL4</sup> mice. G) The expression of various  
996 surface markers by Ly6C- macrophages is represented. Neutros: Neutrophils. Macs: Macrophages.  
997 Mono: Monocytes. Results are A-C, E) pooled from 2 to 4 independent experiments or D, F, G) from a  
998 single experiment. Statistics are A) a two-way ANOVA with a Holm-Sidak's post-test or C-G) Mann  
999 Whitney's test to the WT condition. p<0.05: \*; p<0.01: \*\*; p<0.001: \*\*\*; p<0.0001: \*\*\*\*

1000

1001 **Figure 7: Basophils are more activated and infiltrate more skin wounds in aged mice.** Naïve adult (8  
1002 to 14 weeks old) and Aged (75 to 90 weeks old) C57BL6/J mice were analyzed for their blood content

## Basophils drive wounds resolution

1003 in A) total leukocytes (n=10) and B) proportions off their main leukocyte subsets (n=5). Similarly, the  
1004 surface phenotype of circulating basophils was analyzed in naïve mice (n=5-16), and D) in skin basophils  
1005 24h post wound (n=5-10). Similarly, E) basophil, neutrophil, and Ly6C<sup>+</sup> monocyte skin infiltration were  
1006 compared at different times after wounding (n=13-34) and F) a Basophil to Neutrophil ratio was  
1007 calculated as a percentage for each condition (n=10-34). Results are from B) one single experiment or  
1008 pooled from A) two to C-F) six independent experiments. Statistics are two-tailed Mann-Whitney tests  
1009 to the adult condition. p<0.05: \*; p<0.01: \*\*; p<0.001: \*\*\*; p<0.0001: \*\*\*\*

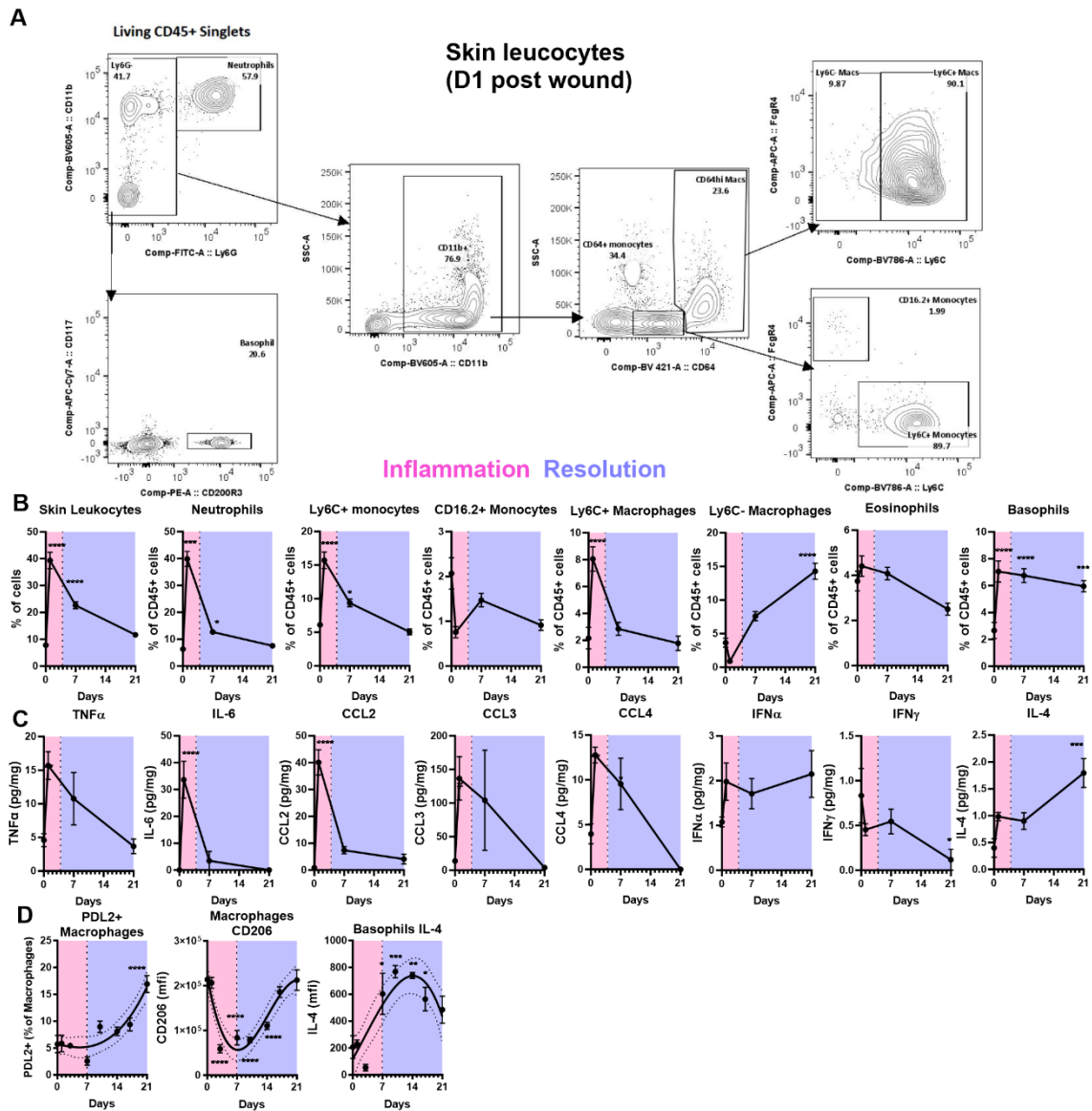
1010

1011 **Figure 8: Basophils infiltrate dorsal wounds of aged mice and show a pro-resolution pro-remodeling**  
1012 **signature.** A previously published scRNAseq dataset (Vu *et al.*, 2022) of dorsal wound cells from young  
1013 (8 weeks old) and aged (88 weeks old) mice at 4 and 7 days post wounding (dpw) was reanalyzed for  
1014 basophils. A) Representative expression of indicated genes after t-SNE unsupervised clustering  
1015 identifies a “Basophil” cluster (MCPT8<sup>+</sup> Kit<sup>-</sup>) from a “Mast cells” cluster (Kit<sup>+</sup> MCPT8<sup>-</sup>) and B) their  
1016 expression of relevant genes. C) The proportion of basophils was quantified in young and aged mice  
1017 from the whole dataset. D) Basophil expression of selected genes from young or aged mice. E)  
1018 Heatmap of the most differentially expressed genes between young and aged mice basophils (4dpw +  
1019 7dpw). F) Enriched gene ontology (GO) of biological processes between young and aged basophils at  
1020 4dpw. G) Circle plots of CellChat-predicted cellular targets of basophils (young + aged), and H) chord  
1021 plots showing the inferred IL-4 or CSF1 signaling networks and cell types involved, in aged mice at  
1022 4dpw. Arrow width represents the predicted strength of the interactions. A, B) v2 + v3 datasets and C-  
1023 G) v3 dataset only. D) Statistics are Mann-Whitney tests. #:p<0.1; \*: p<0.05; ND: Not Detected. IMF:  
1024 Immune modulating fibroblasts. MyoFb: Myofibroblasts. Fb: Fibroblasts. HFDF: Hair Follicle (HF)-  
1025 associated dermal fibroblasts. HF1-3, Basal prolif., Basal and Spinous are keratinocytes subsets. OC-  
1026 like: Osteoclast like. DC: Dendritic cell. APM: Antigen presenting macrophage. NK/TC: Natural Killer and  
1027 T cell cluster.

1028

1029 **Figure 9: Basophils promote the resolution and wound closure in aged mice.** Aged MCPT8<sup>DTR</sup> mice  
1030 were ear punched at Day 0 and depleted in basophils by injection of diptheria toxin at A-C) Day -2, -1,  
1031 and D-G) Day 5, or H-L) at Days 13, 14 and 19 (as represented in H). Total skin leukocyte A, D, I) content  
1032 and B, E, J) composition was analyzed by flow cytometry at Days 1, 7, or 21 as indicated, and the C, F,  
1033 K) phenotype of selected cell types was analyzed. Wound closure was analyzed G) at day 7 in female  
1034 mice (n=10, 9) and L) at day 19 in male mice (n=6, 7). Results come from A, B, C, I, J, K) one single  
1035 experiment or D, E, F) are pooled from two independent experiments. F) Results have been normalized  
1036 on each control condition. A-C) n=16, 18. D, F) n=12-16. I-K) n=8, 8. Statistics are two-tailed Mann-  
1037 Whitney tests. p<0.05: \*; p<0.01: \*\*; p<0.001: \*\*\*; p<0.0001: \*\*\*\*

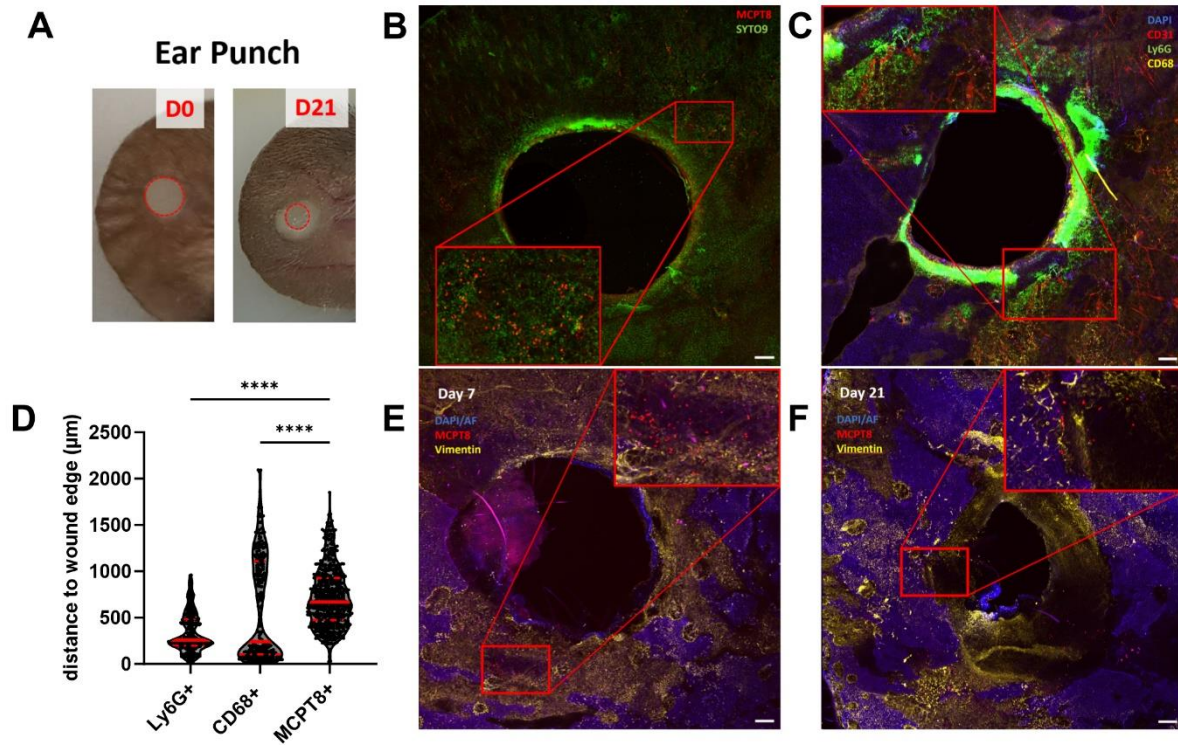
1038



1039

1040 **Figure 1: Basophils are enriched and activated during the resolution of skin wound healing.**

1041

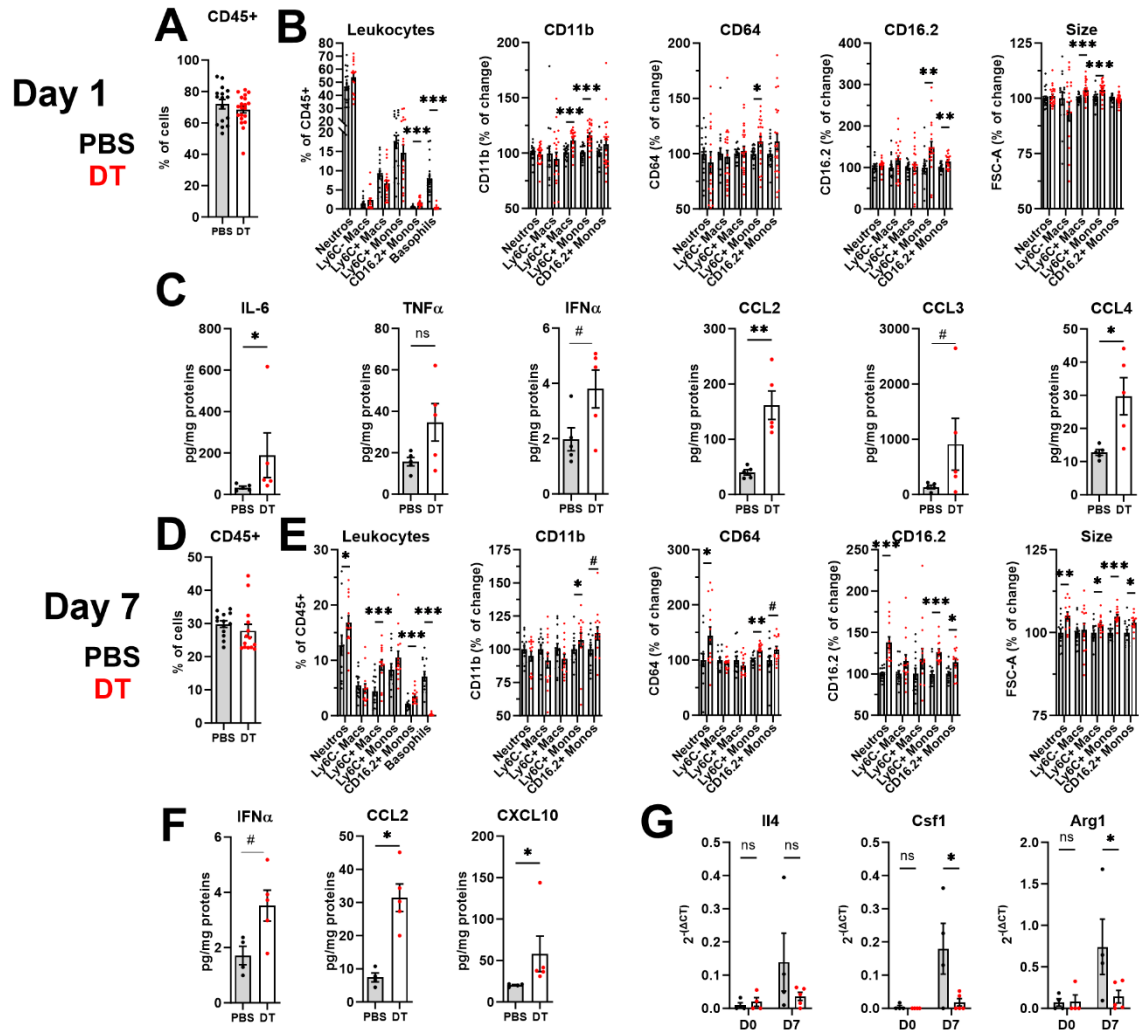


1042

1043 **Figure 2: Basophils infiltrate ear skin wounds during inflammation and resolution**

1044

Basophils drive wounds resolution

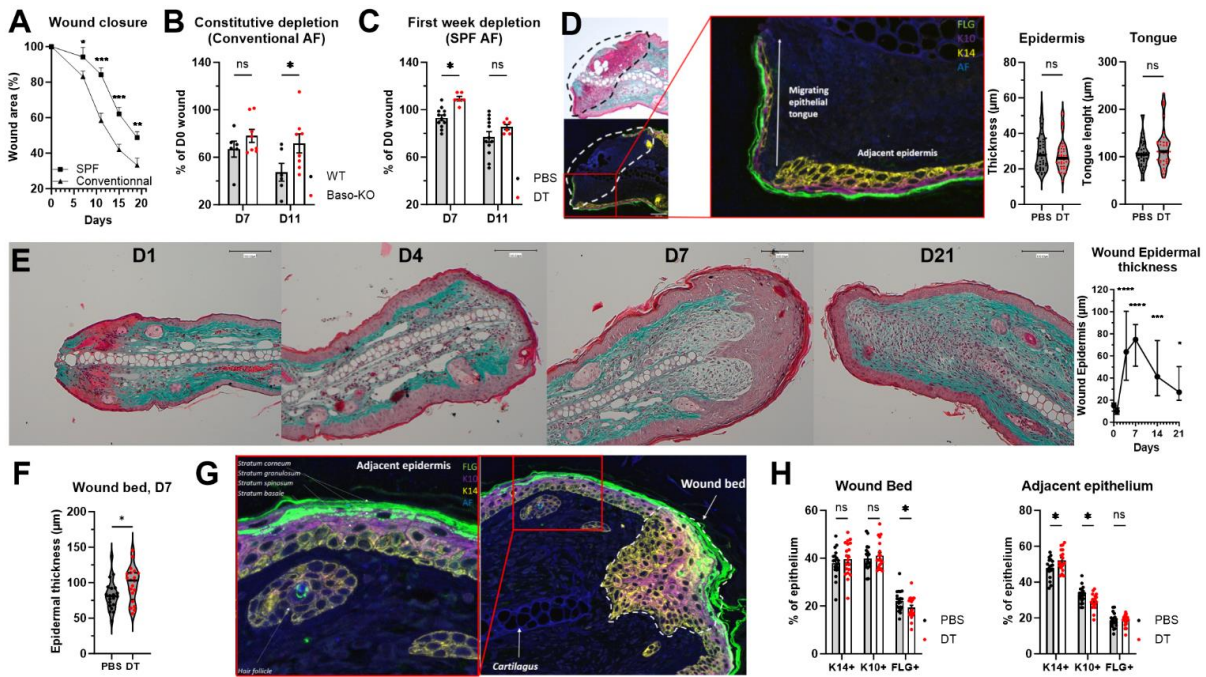


1045

1046 **Figure 3: Basophils dampen activation of pro-inflammatory cells and inflammation during the first**  
 1047 **wound healing**

1048

1049

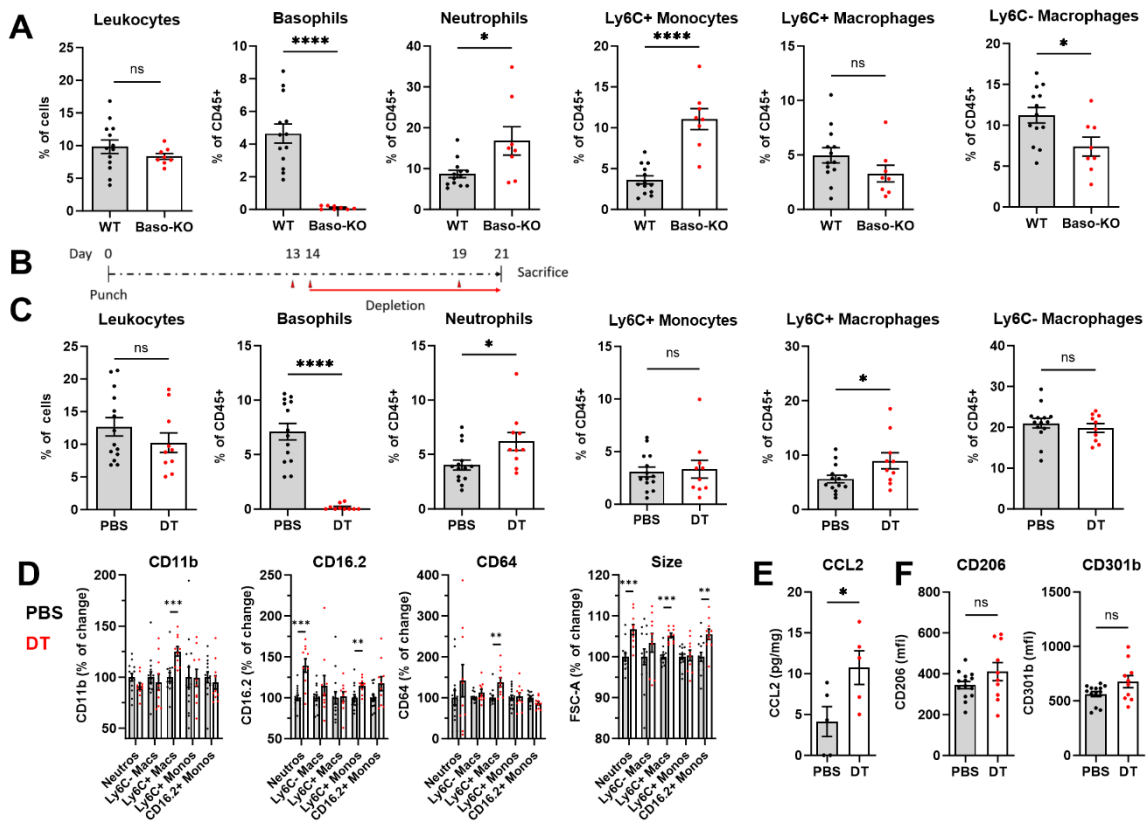


1050

1051 **Figure 4: Basophils accelerate wound healing and the quality of the reepithelialization**

1052

1053

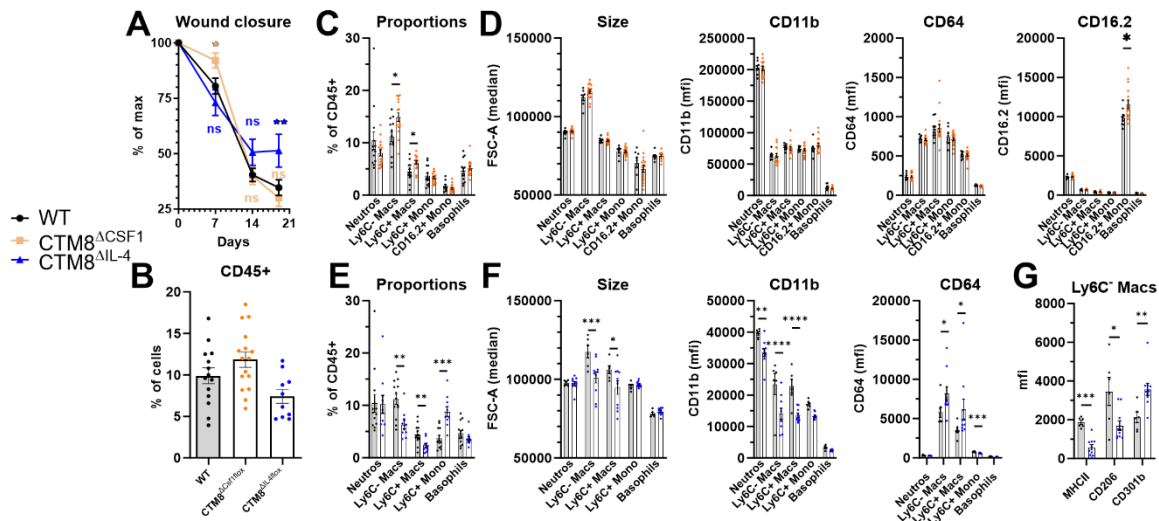


1054

1055 **Figure 5: Basophils dampen the accumulation and activation of pro-inflammatory leukocytes during**  
 1056 **the resolution phase of skin wound healing**

1057

1058

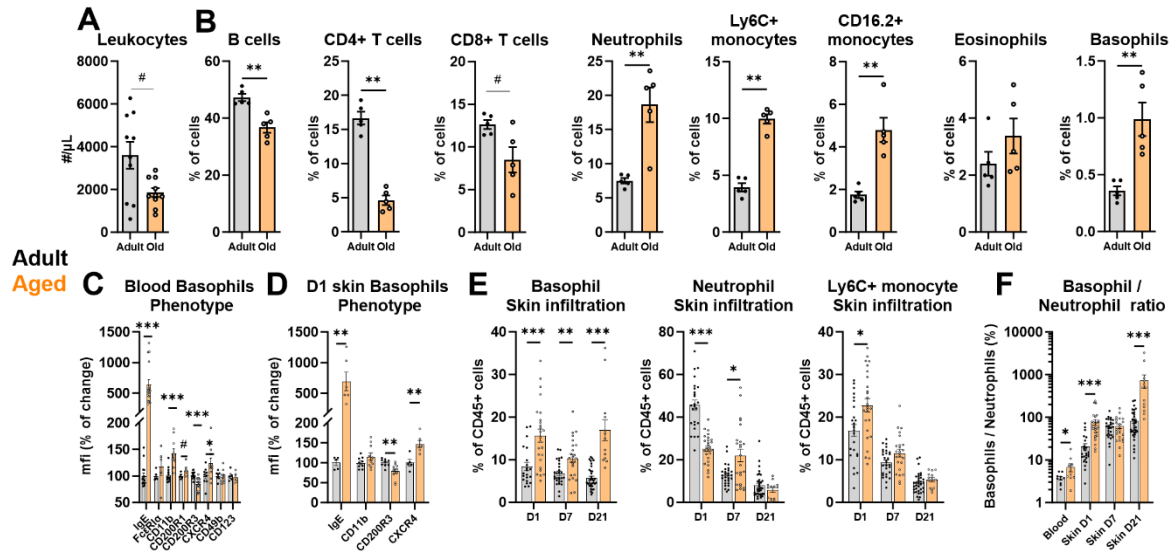


1059

1060 **Figure 6: Basophils CSF1 and IL-4 independently promote wound closure and show complex**  
 1061 **immunoregulatory properties**



Basophils drive wounds resolution

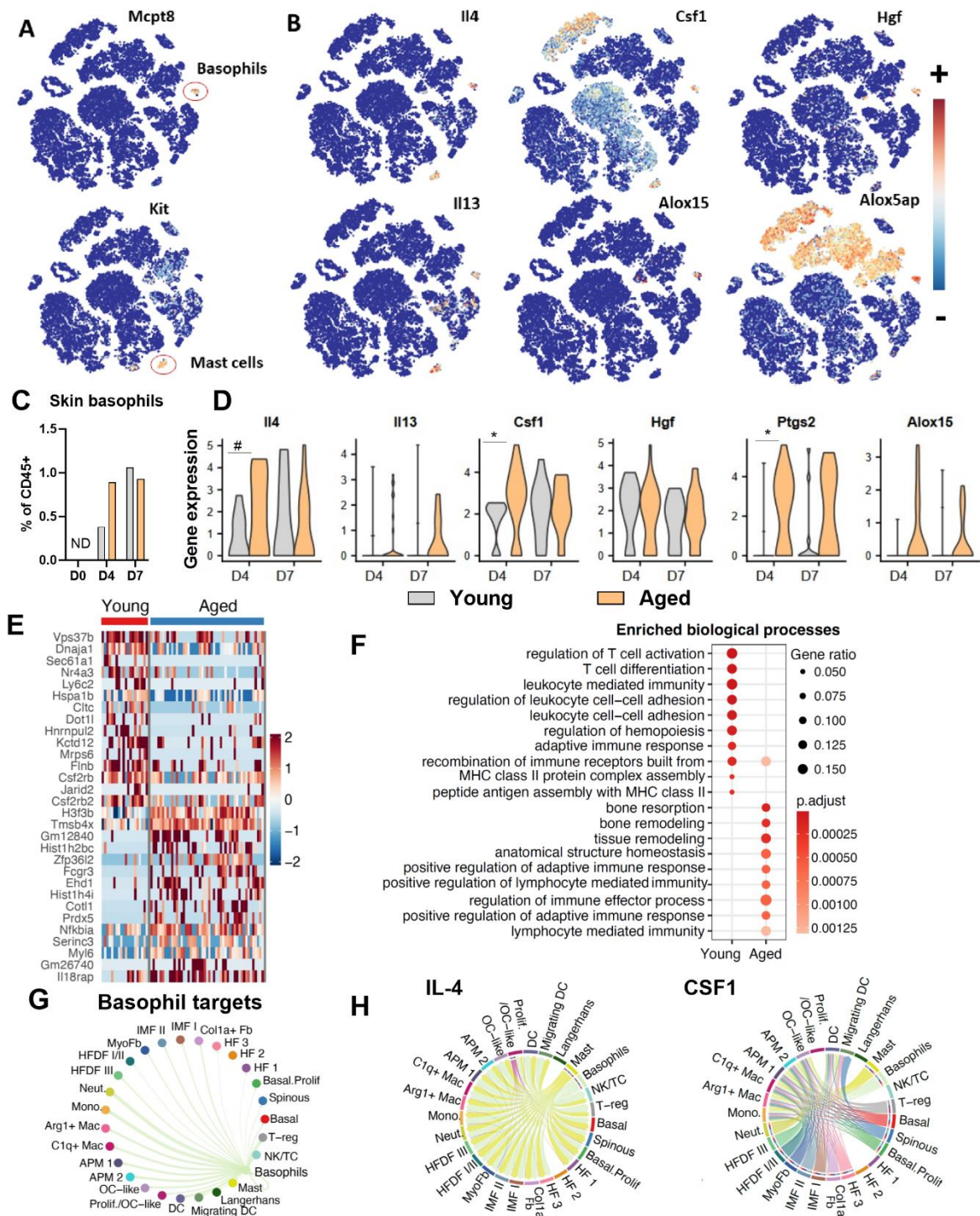


1062

1063

**Figure 7: Basophils are more activated and infiltrate more skin wounds in aged mice**

1064



1065

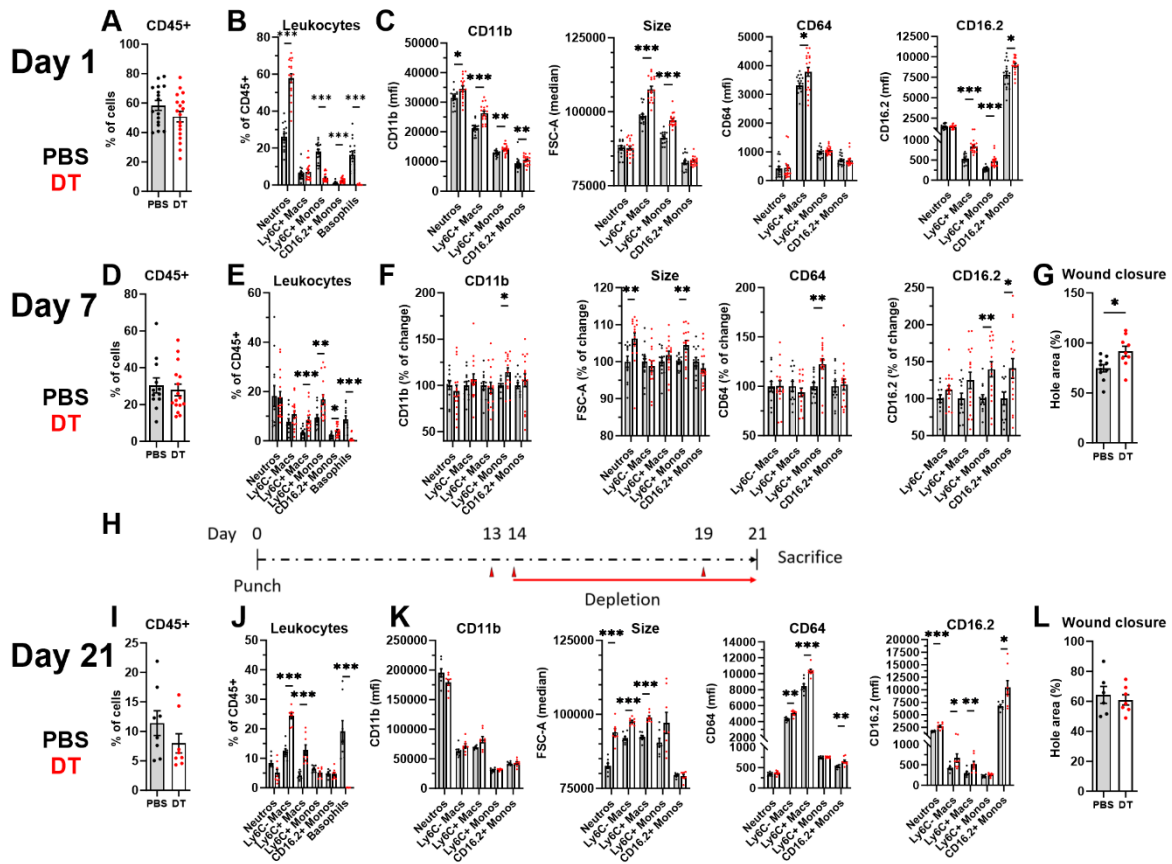
1066

1067

**Figure 8: Basophils infiltrate dorsal wounds of aged mice and show a pro-resolution pro-remodeling signature**

Basophils drive wounds resolution

1068



1069

1070

**Figure 9: Basophils promote the resolution and wound closure in aged mice**